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STAUB EFFECT IN CHILDREN

STUDIES OF THE BLOOD SUGAR REGULATION BY MEANS OF DOUBLE AND TRIPLE GLUCOSE TOLERANCE TESTS

BY

OLLI SOMERSALO

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PREFACE

I obtained the idea of my subject in 1946 while I was working at Kronprinsessan Lovisa's Children's Hospital in Stockholm. I am greatly indebted to its Head, Professor A. Lichtenstein, for his encouraging advice during the initial stages of my study, as well as for his valuable criticism and corrections while this thesis was in preparation.

The work was mainly carried out at the Children's Clinic of the University of Helsinki. I wish to avail myself of this opportunity to express my admiration for and gratitude to its Head, Professor Arvo Ylppö, to whose ardent devotion in the cause of science this work owes its existence. His unfailing interest and numerous valuable suggestions have been of the utmost importance to me.

I am also particularly indebted to Dr. C-E. Räihä for all the help and advice received while the study was in progress.

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I wish to express my deep appreciation to my assistant of long standing, Miss Ulla Fagerholm, who has performed the main part of the glucose estimations.

Finally I thank Mrs. E. Hardén for her English translation of my study.

My work was made possible by the technical help I received from the Research Foundation for Children's Diseases.

Helsinki, in December 1949.

OLLI SOMERSALO

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Introduction

The blood sugar regulation is one of the most investigated sections of Physiology. Especially in recent times many decisive new facts have been discovered. Thus C. F. and G. T. Cori have succeeded in explaining almost completely the mechanism of the insulin action. What we know about the blood sugar regulating factors of the anterior pituitary gland, we owe mostly to Young and Himsworth. Houssey has entirely subverted our knowledge about the mutual relations of the anterior pituitary gland and the pancreas; Long and Lukens have similarly meditied our knowledge of the relations between the anterior pituitary gland and the adrenals. Soskin has founded a school of his own in this branch and has in many respects had a revolutionary influence upon our conception of diabetes.

However, many questions still remain. One of these is Himsworth's insulin kinase theory which has by no means been proved false though it is not necessarily needed to explain the variations of the sugar tolerance. Similarly we do not know whether there are any more hormones regulating blood sugar or substances affecting these hormones or reflexes of the nervous system in addition to those of which we already have knowledge.

One of the most interesting phenomena associated with the regulation of blood sugar is the so called Staub effect. Different theories have been advanced concerning the appearance of this phenomenon. They all are based on the assumption that the elevation of the blood sugar level catalyses its elimination.

While examining in 1946 by means of intra-venous glucose injections, the glucose tolerance of healthy children and of those suffering from coeliac disease, I noticed that the Staub effect was

not manifested in the method I used. Instead it appeared in tolerance tests applied by the mouth, although in that case the blood sugar rose much less. This did not seem to be consistent with the prevailing theory, according to which the Staub effect is due to the elevation of the blood sugar. The question therefore arose whether there is, in addition to the increased blood sugar, also some other agent accelerating its decrease and appearing while the glucose is being absorbed by the intestine or passing through the portal circulation. Such a possibility also suggests itself when one is reading of the agent contained in secretin and promoting hypoglycaemia, the existence of which has been both supported and questioned with equal frequency ever since the discovery of secretin.

The aim of my research is to throw some light on this question by examining the Staub effect in children in double tolerance tests with peroral and intra-venous application.

Bang was the first to find in 1913 that if two successive glucose tolerance tests are performed, the second results in a slighter rise of the blood sugar than the first. This phenomenon was investigated again in 1919 by HAMMAN and HIRSCHMAN and in 1921—22 by STAUB and TRAUGOTT who explained it so that the hepatic cells have a greater capacity to absorb the glucose from the blood during the second test.

This »Bahnung» of the intermediary carbohydrate metabolism, which also appears in adults if glucose is introduced by two intravenous injections, has been subsequently explained in different ways. To clarify the various theories, I shall attempt to give below a short review of our present-day knowledge concerning the blood sugar regulation, with special consideration of the agents affecting the glucose tolerance curve, in particular of such mechanisms, if any, which may be involved in the appearance of the Staub effect.

Glucose absorption from the intestine.

Carbohydrates are absorbed as monosaccharides. According to the modern opinion, a small part of the absorption takes place by osmosis, but is chiefly brought about by the activity of the intestinal wall in such a way, that in the luminal end of the cell the hexose molecule is combined with phosphoric acid to form an ester, and at the basal end of the cell this compound is broken up and the hexose molecule passes through the capillary wall into the blood. The mechanism has not yet been fully elucidated; it appears however that the phosphoric acid »transports» the glucose molecule through the cell of the intestinal wall. The velocity of the glucose absorption in man is not fully known, but it is assumed that the maximal velocity is approximately 2 g/min., and the absorption velocity independent of the quantity administered, at any rate if it is 1 g/kg or more (LUNDSGAARD 1948).

Blood sugar regulation.

Liver.

The importance of the liver to the blood sugar regulation was already suspected by CLAUDE BERNARD in 1850. In the fasting animal the liver is the sole source of blood sugar. (MANN in 1927, SOSKIN in 1927, 1941). After hepatectomy the blood sugar falls abruptly and the test animal dies of hypoglycaemic convulsions. Naturally there is still glycogen in the muscles also after the removal of the liver, but it cannot be converted into glucose. Epinephrine does

not prevent the fall of the blood sugar after hepatectomy, (SOSKIN in 1927) nor do the extracts of the anterior pituitary gland (HOUSSAY in 1936). Thus, during fasting time, the liver continues to secrete glucose into the blood. If on the other hand glucose is absorbed from the intestine or administered parenterally, this secretion stops and the liver begins to store glucose instead. SOSKIN, ESSEX, HERRICK and MANN obtained in 1938 a better knowledge of this regulatory activity of the liver. They injected glucose intra-venously into the dog and determined its amount in the portal and the hepatic vein both at the time of injecting and after it. They noticed that the liver, which before the injection had secreted glucose into the blood, began already at the time of the injection to absorb it strongly from the blood, until normal blood sugar values were again restored. After that the liver neither absorbed nor secreted any glucose for approximately one hour. During this time the sugar content of capillary blood fell below normal (consecutive hypoglycaemia), since extrahepatic tissues naturally used up glucose; hypoglycaemia disappeared first after the liver began again to secrete glucose into the blood. (Fig. page 50). A similar experiment was performed by CHERRY and CRANDALL in 1937 by administering glucose to the dog by the mouth.

This regulatory activity of the liver is of course undisturbed only as long as the normal endocrine balance prevails, but it can evidently function fairly independently as well, emancipated to a great extent at least from the pancreas and the pituitary gland, since a dog with both these organs removed, yields normal glucose tolerance curves, apart from their having a higher starting point than in the normal dog (SOSKIN, MIRSKY, ZIMMERMAN and HELLER, in 1935—36). If the liver of the healthy dog begins storing glucose when the blood sugar surpasses 0.1%, in such a »Houssay-dog» the same regulation begins to function only at a much higher blood sugar level. The great importance of this hepatic regulation mechanism is also demonstrated by the circumstance that if the liver is removed and the blood sugar subsequently maintained on the normal level by hourly infusing into the animal about 0.25 g glucose per kg of body weight, this quantity being used up by the tissues, a diabetic glucose tolerance curve is obtained (SOSKIN, ALLWEISS and COHN in 1934). Therefore, extrahepatic tissues are unable

— even when a normal endocrine balance prevails — to compensate for the glucose absorbing capacity of the liver.

This capacity of the liver to retain sugar from the blood is the greater, the higher the blood sugar level. In the same way, the formation of glycogen in the liver is the more accelerated, the higher the blood sugar elevation. The quantity of the glycogen formed is more dependent on the blood sugar level itself than on the glucose quantity administered (CORI and CORI in 1929).

Extrahepatic tissues.

The role played by the extrahepatic tissues in the blood sugar regulation is much smaller than that of the liver. While a high hyperglycaemia is prevailing, the glucose is naturally being diffused into the extracellular fluid, so that its sugar concentration finally becomes identical with that of the blood. Yet this is, partly at least, a passive process, through the osmosis, in contradistinction to the liver whose regulation is active and where the sugar concentration at the end of the infusion can be much higher than in the blood. However, the significance of extrahepatic tissues, particularly the muscles, in the blood sugar regulation is not only confined to the glucose diffusion into them, i.e. to their activity of storing sugar. It has been demonstrated in fact (SOSKIN and LEVINE in 1937) that the sugar utilisation of an eviscerated dog improves with the elevation of the blood sugar level. Likewise HECHTER, LEVINE and SOSKIN found in 1941 in vitro that the formation of glycogen in muscles is directly comparable to the sugar concentration. It has been said already that glycogen in the muscles cannot be directly converted into blood sugar. This is an indirect process by way of the lactic acid, which is again synthetised to glycogen in the liver, but the whole proceeding takes such a long time, that it cannot be of any great significance for the regulation of blood sugar under normal conditions.

The significance of the endocrine glands in the blood sugar regulation may by summarised as follows:

Pancreas.

The effect of insulin has been examined:

1. in vitro
2. by injecting it into depancreatised animals and diabetics
3. by injections into healthy individuals

In vitro tests were performed by e.g. HECHTER, LEVINE and SOSKIN in 1941. They were able to demonstrate that insulin increased the glycogen level in muscles while the medium glucose concentration was low, but the higher the medium concentration, the easier the formation of glycogen also without the help of insulin, and at a glucose level of 0.4%, insulin did not promote anymore the muscular capacity of glycogen formation.

It could not be proved by means of perfusion tests that insulin had any effect on the glycogen formation in the liver.

When injected into man or animals, insulin always brings about hypoglycaemia, also if hepatectomy had been previously performed on the animal and a spontaneous fall of the blood sugar level prevented by constant glucose infusions (MANN and MAGATH in 1923). It appears evident that insulin has a direct effect on the tissues, so that they absorb the sugar from the blood more rapidly. This was also proved by SOSKIN and LEVINE in 1937 and 1940 in both depancreatised and non-depancreatised dogs on whom hepatectomy had been performed. Insulin affects the liver in such a way that the hepatic secretion of sugar into the blood decreases, as shown by VON ISSEKUTZ and SZENDE in 1934, BEST in 1934 and SOSKIN in 1940.

The insulin hypoglycaemia is therefore due to two main phenomena — an increase of the tissular capacity to absorb glucose, and a decrease of the hepatic secretion of the sugar.

It must be borne in mind that the effect of hypoglycaemia on the glycogen formation, the sugar utilisation and the glyconeogenesis is opposite to that of insulin, and therefore hypoglycaemia due to insulin can wholly or partly eliminate the insulin effect in this

respect. Furthermore, hypoglycaemia promotes the secretion of the antihormones of insulin — epinephrine and the sugar hormones of the anterior pituitary gland.

As evidenced by the in vitro test reported above, the formation of glycogen is possible also without insulin, provided the glucose concentration is high enough. MAJOR and MANN in 1932, demonstrated this on a depancreatised dog who was, five days after withdrawal of insulin, capable of forming glycogen in both skeletal muscle and liver, although at a rate far below that of a normal dog.

There is much controversy as to whether insulin helps to oxidise the glucose. The »non utilisation theory» advanced by MINKOWSKI, which was the sole prevailing until recent times, considered that the oxidation of glucose in diabetes was entirely inhibited without insulin. The modern opinion, especially propounded by SOSKIN — the »overproduction theory» — is based on the assumption that a diabetic has a combustion of glucose similar to or higher than the normal individual, but in the absence of insulin, there is an increased glyconeogenesis.

Since hepatectomy performed on depancreatised animals causes a fall of the blood sugar and death from hypoglycaemia, in exactly the same way as when normal animals are subjected to hepatectomy (MANN and MAGATH in 1923), it appears that a diabetic's tissues are using up the glucose; although, strictly speaking, the passing of glucose from the blood into the muscles need not necessarily mean that it undergoes combustion there. Yet SOSKIN and LEVINE were able to show in 1937 that a depancreatised dog utilises glucose also without insulin, and that the utilisation is directly comparable to the blood sugar elevation, exactly as in healthy animals. »Within a wide range of blood sugar values the diabetic dog utilizes less sugar than does the normal dog at any particular blood sugar level. But, above certain high levels this difference disappears and both types of animals use the same amounts of carbohydrate at the same blood sugar levels.»

The absence of insulin has a deteriorating effect on the utilisation, but it is compensated by the rise of the blood sugar. LUNDSGAARD says as well in his textbook on physiology 1948/p. 450: »It should be understood that one can very well assume the carbo-

hydrate combustion to be inhibited, and still take into account that the carbohydrate combustion proceeds in its normal value in the extrahepatic tissues of a diabetic organism.»

A diabetic who is not administered insulin, excretes abnormally large amounts of nitrogen in the urine (LUSK, MACLEOD). The in vitro work of BACH and HOLMES in 1937 supports the assumption that insulin has a proteinpreserving effect on the diabetic organism: glyconeogenesis diminishes under the influence of insulin.

According to the above, insulin affects the diabetic as follows:

1. Accelerates the formation of glycogen in muscles and liver and prevents glycogenolysis in the liver. This occurs despite the fall of blood sugar.
2. Prevents glyconeogenesis from proteins, also irrespective of the fall of blood sugar.
3. Promotes utilisation, if the blood sugar is maintained at a high level.

It seems that insulin administered to healthy individuals has a lesser influence on the above-mentioned processes.

According to BODO and NEUWIRTH in 1933, CORI in 1925 and 1929, insulin reduces the glycogen amount in the liver of a healthy animal.

In the normal animal, the effect of insulin is to increase glycogen in the muscles. (CRUICKSHANK and STARTUP in 1933).

Unreliable and contradictory results have been obtained on the effect of insulin on the protein metabolism in normal animals. It seems however that the amino acid concentration in the blood decreases under the influence of insulin (KERR and KRIKORIAN in 1929), but this does not occur in absence of the adrenal medulla (LUCK and MORSE in 1933). Therefore this insulin effect is probably due to the epinephrine secretion produced by insulin hypoglycaemia.

There are several works, according to which insulin promotes the oxidation of carbohydrates in the normal individual (BEST, DALE, HOET and MARKS in 1926, WIERZUCHOWSKI in 1931, CORI in 1931).

Soskin and Levine performed carbohydrate balance tests after insulin administration in 1937 and 1940 by registering the decrease of the total carbohydrate amount in the experimental animal during the test, also taking into account the formation of sugar by the liver. They arrived at the result that insulin does not increase the rate of utilisation in normal animals, even if a fall of the blood sugar level is prevented by glucose infusions.

To summarise, it can be said therefore of the insulin effect on the normal:

1. Insulin accelerates the formation of glycogen in the muscles. Does not affect the glycogen formation in the liver, or reduces it even if the blood sugar level is not allowed to fall.
2. Possibly decreases glyconeogenesis.
3. The question whether insulin promotes utilization, is not yet definitely settled.

Soskin's theory would signify that a normal animal has an amount of insulin in its organism which is optimal from the point of view of sugar utilisation and of protein metabolism, and therefore additional insulin only promotes the storing-up.

The regulation of insulin secretion.

Because the blood sugar level begins to rise immediately after pancreatectomy, there must be a continous secretion of insulin from the pancreas. This is further evident from the circumstance that a prolonged constant insulin infusion is necessary to maintain a normal blood sugar level in a depancreatised dog also when it is fasting (Soskin and Allweiss in 1934). Without this insulin infusion its glucose tolerance curve becomes diabetic, but a constant infusion suffices to keep it normal, even to produce a Staub effect (Soskin, Allweiss and Cohn in 1934).

Therefore, even if additional insulin is not necessary to produce a normal glucose tolerance curve and Staub effect, it yet appears certain that the secretion of insulin is heightened during hyper-

glycaemia or after the injection of carbohydrates (FOSTER in 1923, STAUB 1926, GRÄFE and MEYTHALER 1927—8, HOUSSAY 1937).

On the other hand, it seems in the light of HIMSWORTH's experiments in 1935—36 that the intensity of a basal insulin secretion between meals is independent of the diet.

Difference of opinion has been prevailing whether hyperglycaemia stimulates the pancreas directly or whether its effect is transmitted by the autonomic nervous system, or if both these mechanisms are responsible.

MINKOWSKI's and HÉDON's transplantation experiments have proved conclusively that the nervous system is at least not absolutely necessary for the normal insulin secretion. They removed the pancreas from their experimental animal and transplanted it under its skin in such a way that the blood vessels alone connected the organ with the animal. The dog did not develop diabetes.

HOUSSAY in 1937 is also of the opinion that the insulin secretion is essentially humoral, since pancreatic denervation reduces the tolerance but slightly and since the pancreas grafted into the neck functions normally without extrinsic nerves. Grafting the pancreas was performed in such a way that the pancreaticoduodenal artery is connected with the carotid artery, and the pancreatic vein with the jugular vein. Yet the nervous system plays some part in the insulin secretion since, when the pancreas is denervated, the intravenous glucose tolerance curve is somewhat slower and hypoglycaemia subsequent to the insulin injection is a little slower to disappear than normally. Therefore, according to HOUSSAY, the vagus is cooperating with the humoral mechanism, increasing the secretion of insulin during the hyperglycaemia and decreasing during the hypoglycemia. But the humoral mechanism is the essential.

Another argument in favour of chiefly the humoral mechanism is the observation that if 3 pancreases are transplanted into a normal dog, one into the neck and two into the inguinal folds — the blood sugar remains unchanged (HOUSSAY 1937). They therefore regulate their secretion so, that they jointly secrete the same amount of insulin as one pancreas of the normal dog.

The following test performed by GRÄFE and MEYTHALER in 1927 and 1928 also points in the same direction. If glucose is injected into the pancreaticoduodenal artery, hypoglycaemia is the result, in contradistinction to the effect, if glucose is injected into any other artery. The glucose has therefore stimulated the pancreatic insulin production.

ZUNZ and LA BARRE have attempted to prove by performing vein anastomosis (pancreaticojugularis) between two or three dogs, preserving the vagus in one experiment and cutting it in the other, that the regulation of insulin secretion occurs mainly through the vagus.

GAYET in 1929 repeated this test of ZUNZ and LA BARRE and obtained quite a different result. He was unable to observe any stimulating effect of the vagi on the insulin secretion.

It appears as if the humoral mechanism had been conclusively proved in the regulation of the insulin secretion. This is probably the most important mechanism and is sufficient in itself to regulate the normal sugar metabolism.

GAYET entirely denies the existence of the nervous mechanism. It seems however that this is venturing too far in the light of those numerous experimental researches, according to which vagal stimulation produces hypoglycaemia. SOSKIN is of the opinion that »vagal stimulation of extra insulin secretion acts as a restraining counter-regulation in limiting the hyperglycaemic effects of the adrenal medulla and the sympathetic nervous system.«

Latency period of insulin.

An intra-venous insulin injection is followed by a period of latency of about 2 to 10 minutes. The blood sugar begins to fall only after the lapse of this time. The difference of the sugar concentration prevailing in the arterial and venous blood, the so-called arterio-venous difference, remains constant during this time as well, after which it increases as a mark of the peripheral tissues having begun to absorb the sugar from the blood with greater velocity.

The latency period can be imagined, according to HIMSWORTH, in 1933—34 to be due to the following causes:

1. The insulin attains within this time its site of influence in the tissues.

2. The insulin is inactive when it is being injected and also when it reaches the tissues. This may be due to the tissues containing some agent with which the insulin must form a compound before it becomes active (1934).

3. The tissues contain something inhibiting the effect of insulin, until the latter has neutralised it (1938).

According to HIMSWORTH, the first possibility is not very probable, since the blood circulation time is about 2 minutes, the latency period however often being 8 to 10 min. If the latency period were due to the penetration of the cells by insulin, it ought to be shortened if additional insulin is administered. Yet the latency period is independent of the dose. If insulin has to pass through the liver, it is inconceivable that insulin should function without the liver.

It therefore appears probable, in accordance with HIMSWORTH, that the period of latency is due to the organism being insensitive to insulin during the first minutes (possibilities 2 or 3).

Sensitivity to insulin.

It is well known that the sensitivity of the organism to insulin varies greatly. The effect of insulin is much stronger after a diet rich in carbohydrates than after a diet deficient in them (HIMSWORTH in 1934). Moreover, the latency period is shortened up to 2 minutes by a diet rich in carbohydrates. Similarly to the effect of this diet, administration of glucose also intensifies the sensitivity to insulin. HIMSWORTH injected intravenously 1 gr glucose into the dog 4 times at an interval of 35 minutes and the insulin tolerance test was performed 40 minutes after the blood sugar had gone back to normal subsequent to the last injection. It was observed that the period of latency had been shortened and hypoglycaemia was stronger than in the insulin tolerance test made before the glucose injections.

Fasting has the same effect as a diet deficient in carbohydrates, i.e. it reduces the sensitivity to insulin (TITTSO in 1925).

In the same way as the diet, the actual elevation of the blood sugar level also affects the sensitivity to insulin: the higher the blood sugar level, the greater the sensitivity to insulin. Very much more insulin would be required to lower the blood sugar from 50 to 40 mg % than from 90 to 80 mg % (HIMSWORTH in 1932, 1933—34).

The same factors which affect the sensitivity to insulin, also affect the sugar tolerance. ADLERSBERG and PORGES in 1926, noticed that a diet rich in carbohydrates increased the glucose tolerance and vice versa. SWEENEY in 1927, observed that fasting and fatty diet reduce the tolerance, carbohydrate diet improves it and the effect of proteins lies in between. HIMSWORTH observed in 1935—36 that if the proportion of carbohydrates and fats in the diet remained constant and the amounts of both were increased, the glucose tolerance improved. If, on the other hand, the amount of fat was increased while the carbohydrates remained unchanged, the tolerance persisted as before. Therefore he maintains that glucose tolerance is solely dependent on the absolute carbohydrate amounts contained in the diet. C. W. ROSS in 1936—37 also supports this opinion. The correlation between the glucose tolerance and the insulin sensitivity was studied by HIMSWORTH in the following way: 7 diets of 50—500 gr carbohydrates per day were administered, each for a time of one week. At the end of each week the sensitivity to insulin was determined from the area of the insulin tolerance curve, and on the following day — the glucose tolerance from the area of the peroral glucose tolerance curve. The amount of proteins and calories remained unchanged for the whole time, the fat amount was accordingly reduced. He observed that the glucose tolerance and the sensitivity to insulin rose to exactly the same level. From this he draws the conclusion that the same sugar dose has produced an equal amount of insulin during each diet. Since, if the insulin secretion had increased on a diet rich in carbohydrates, this increased secretion plus the heightened sensitivity would have caused together a greater rise of the sugar tolerance than of the sensitivity to insulin.

Therefore, according to HIMSWORTH, the carbohydrate diet continually intensifies the sensitivity to insulin, but it increases its secretion only for the period of hyperglycaemia. On the disappearance of hyperglycaemia, the insulin secretion again resumes the same speed as before, and the speed of secretion is independent of the diet.

We do not yet know certain what causes the sensitisation to insulin in the organism. As already said on p. 18, two possibilities can be considered:

1. To exercise its effect, insulin needs some other agent, an activator. This may be generated in the liver when the sugar is being introduced into the organism either the enteral or the parenteral way.

2. Some agent, the »inhibitor», prevents the effect of insulin. This substance would decrease under the conditions named.

As an argument for the first possibility HIMSWORTH reported in 1933 the circumstance that increasing the dose of insulin above a certain maximum does not accelerate the manifestation of insulin activity. »This is explained on the grounds that the amount of active insulin produced per unit of time is limited by the concentration of the activator present in the tissues, any excess of insulin remaining temporarily outside of the scope of the reaction. The maximal dose of insulin is the amount required to bring into action all the activator present in the tissues».

The sensitivity to insulin did not rise after the insulin administration, contrary to what happens after the glucose administration. HIMSWORTH in 1933—34, draws the conclusion that the stimulation required to produce insulin kinase is hyperglycaemia. Another circumstance speaking perhaps for the activator theory is that the insulin effect continues as before immediately after the removal of the liver, but this effect stops after the lapse of 6—8 hours (MARKOVITZ, MANN and BOLLMANN in 1929), i.e. the insulin activator decreases with the decrease of hepatic tissue. A further support for the insulin activator is provided by the reduced sensitivity to insulin during disorders of the liver, whereas this sensitivity increases during the period of regeneration (ALTHAUSEN and THOENES in 1932).

The activator theory can perhaps partly explain also the tests reported below concerning the possible rôle of the duodenum in the carbohydrate metabolism.

The second possibility, the inhibitor theory, has gained ground particularly after YOUNG's hypophysis studies. According to it, both the latency period and the variations in sensitivity are due to some agent inhibiting the insulin effect. The theory concerning this is presented in the following paragraph.

Hypophysis.

Hypophysectomy produces, beside trophic disorders in other glands of internal secretion, also immediate changes in the intermediary metabolism. The immediate changes are, according to SOSKIN and LEVINE, the following:

1. The blood sugar decreases by about 20—30 mg % (SOSKIN et al. 1935, HOUSSAY 1936).
2. Fasting rapidly produces a strong fall in the blood sugar (SOSKIN, MIRSKY, ZIMMERMAN and CROHN in 1935), contrary to the state of things in the normal, who can fast for a long time without any noteworthy fall in the blood sugar.
3. The glucose absorption deteriorates.
4. The nitrogen excretion into the urine decreases (HOUSSAY). This is at least partly due to thyroid atrophy.
5. The basal metabolism is delayed.
6. The sensitivity to insulin increases.
7. The serum potassium amount decreases.
8. The amount of reduced glutatione in the blood, liver and muscles decreases (LEVINE, HECHTER, GROSSMAN and SOSKIN in 1939).
9. The growth is arrested.

We have at present no knowledge of all factors, whose absence produce the changes referred to above, nor, in many cases, whether their effect is direct or through the intermediary of some other organ. FRANK in 1949 assumes the following 6 principles: growth

hormone, adrenotropic, (JENSEN and GRATTAN in 1940) thyrotropic, lactogenic (FRAENKEL-CONRAT in 1943), gonadotropic, diabetogenic, one fraction of which could possibly be identical with YOUNG's glycotropic factor (YOUNG in 1938).

If the pituitary is removed from a dog affected with pancreatic diabetes, (HOUSSAY in 1931 and 1936) it survives for a considerably longer time without insulin and its glucose tolerance is almost normal. According to SOSKIN, MIRSKY, ZIMMERMAN and HELLER in 1936, the Staub effect is absent in a hypophysectomised dog. The HOUSSAY dog is highly sensitive to insulin (HOUSSAY in 1936), which proves that the hypophysis contains some factor or factors with an effect opposed to that of insulin or inactivating it. HOUSSAY assumes that pituitary secretion stimulates gluconeogenesis. The work of WELLS et al. on rats supports this, but according to them, the gluconeogenetic influence of the thyroid gland and of the adrenal cortex are each partly responsible for the total effect of the anterior pituitary. On the other hand, results obtained by FISHER, RUSSELL and CORI in 1936 are inconsistent with HOUSSAY's theory. They assume instead that the pituitary gland has an inhibitive effect on the oxidation of carbohydrates.

RUSSELL in 1938 came to the same conclusion, that the HOUSSAY dog had a better sugar utilisation than a diabetic animal. This is strongly contested by SOSKIN, LEVINE and LEHMAN in 1939. »It was found that the extrahepatic tissues of hypophysectomised dogs utilised sugar at subnormal rates and that the pituitary extract did not influence the rate of sugar utilisation by extrahepatic tissues of normal dogs.» HIMSWORTH and SCOTT in 1938 assumed that the glycotropic factor functioned as a peripheral anti-insulin. They injected pituitary extract into the hepatectomised dog. It did not in any way affect the fall of the blood sugar which always occurs after hepatectomy, but it inhibited the influence of insulin accelerating this fall. It is claimed by these authors in 1938 that hypophysectomy produces a decrease in difference for the glucose tolerance between a diet rich in carbohydrates and deficient in them, but this difference does not disappear entirely. The Staub effect was clearly manifested in their tests on a carbohydrate diet although the hypophysis was removed. On a fat diet it was also manifested, but not well, whereas in the normal animal it is reversed

after a fat diet (HIMSWORTH in 1934). A treatment with the glycotropic factor rendered the Staub effect negative, although the dog was kept on a carbohydrate diet. This means that the glycotropic factor transformed the Staub effect into what it is in the normal dog subsisting on a diet deficient in carbohydrates. This shows, according to HIMSWORTH and SCOTT, that if carbohydrates are cut down in the diet, the pituitary gland increases its secretion, thus bringing about a diminished glucose tolerance and a weakened sensitivity to insulin. SOSKIN is of the same opinion in 1936. The insulin kinase theory would therefore, according to them, become superfluous. YOUNG in 1938 claims that the glycotropic factor causes a slight weakening of the tolerance in intravenous glucose tolerance tests and makes the intra-venous Staub effect reversed in normal rabbits, in the same way as in HIMSWORTH's and SCOTT's tests on hypophysectomised rabbits. Similarly to HIMSWORTH and SCOTT, he also assumes that the glycotropic factor directly antagonises the action of insulin both in the liver and in the peripheral tissues.

According to BARNES and DIX in 1936, the insulin sensitivity of the hypophysectomised animal is due to the glycogen being then much more stable than in the normal, and therefore the epinephrine secreted in hypoglycaemia does not have any effect (FRANK in 1949).

JACOBS in 1938 as well as LEHMAN and SCHLOSSMANN in 1938 have found that the sulfhydrylgroups (reduced glutatione and the protein SH-groups) inactivate insulin. On the other hand, there is much less reduced glutatione in the liver of a hypophysectomised than of a normal animal (LEVINE, HECHTER, GROSSMAN and SOSKIN in 1939). Of course, this circumstance also argues in favour of the assumption that a heightened sensitivity to insulin is due to a delayed inactivation (SOSKIN and LEVINE in 1946).

The variations in the insulin sensitivity and the glucose tolerance can be explained, on the basis of the above, to a great extent with the help of the hypophysis, but not fully. The specific dynamic action of glucose is not due to the pituitary gland — Chambers et al. in 1935 have shown that in hypophysectomised dogs glucose caused the usual rise of RQ and that after starvation these dogs show the same reduced rise of RQ as starving normal

dogs. In HIMSWORTH's opinion, the Staub effect is also independent of the hypophysis, at least partly, whereas, according to SOSKIN, this is due to a diminished pituitary secretion during hyperglycaemia.

Adrenals.

1. *Adrenal cortex*

If the adrenal cortex is removed, changes are generated in the carbohydrate metabolism, which greatly resemble changes subsequent to hypophysectomy. The removal of the adrenal cortex improves diabetes in exactly the same way as the removal of the hypophysis (LONG and LUKENS in 1936). Just as in hypophysectomy, adrenalectomy also produces strong hypoglycaemia during fasting, a decrease in the nitrogen excretion and increase in the sensitivity to insulin, as well as a weakening of absorption. Different workers are fairly unanimous in the opinion that the primary metabolic functions of the adrenal cortex are concerned with hepatic gluconeogenesis (SOSKIN and LEVINE in 1946, KAPPERT in 1947). The assumption that the cortical portion has a glycogen preserving influence, is supported by the work of COREY and BRITTON in 1941. They obtained a strong increase of glycogen in liver perfusion tests by adding adrenal cortical extract besides glucose to the perfusion fluid. Insulin did not exercise any effect.

The anterior pituitary partly produces its effect through the adrenal cortex, as surmised by WELLS with regard to gluconeogenesis and by LONG and LUKENS with regard to the diabetogenic factor.

Similarly to the state of things in hypopituitarism, according to SOSKIN, the Staub effect often remains non-apparent in insufficiency of the adrenal cortex as well (KAPPERT in 1947).

2. *The adrenal medulla and the sympathetic nervous system.*

A fall in the blood sugar produces a secretion of epinephrine, according to CANNON in 1924 if the blood sugar decreases to 70% of the normal. An epinephrine injection causes the blood sugar

to rise, due to the breaking-up of glycogen in the liver, and also produces a rise of the lactic acid, which results from the breakdown of muscle glycogen (YOUNG in 1936).

Hyperglycaemia due to epinephrine does not accelerate the oxidation of glucose (BORNSTEIN in 1921) and, as shown by CORI and CORI in 1929, glucose alone produced a greater glycogen formation than if administered together with epinephrine, although the blood sugar was then twice as high.

According to SOSKIN, epinephrine and the sympathetic nervous system are not significant for the regulation of the blood sugar. They are only an emergency safeguard against hypoglycaemia that is too rapid or too severe to be adequately handled by other mechanisms. The blood sugar regulation actually remains sufficient after denervation or destruction of the adrenal medulla as well as after total sympathectomy and after the removal of the coeliac ganglion (BROUHA, CANNON and DILL in 1939). After these operations the glucose tolerance improves and the sensitivity to insulin increases somewhat (TAKATS and CUTHBERT in 1932).

The thyroid gland

The rôle of the thyroid gland in the glucose tolerance has been deprecated, according to MARINE in 1922 for the reason that thyroidectomy has been incompletely performed.

The thyroid is known to have at least the following effect on the carbohydrate metabolism: the thyroid hormone accelerates the absorption of glucose and renders the glycogen in the liver less stable. Therefore in hyperthyroidism a peroral diabetic glucose tolerance curve is found, the curve being low in hypothyroidism. The thyroid hormone stimulates glyconeogenesis and it is probable that this effect of the pituitary partly occurs through the thyroid (SOSKIN, LEVINE and HELLER in 1939). In hyperthyroidism the carbohydrate utilisation is abnormally rapid, coincident with the increased amounts of glucose entering the blood from the gastrointestinal tract and from the liver.

Surmises on the role of the duodenum in the carbohydrate metabolism

There has been much controversy as to whether it is possible to isolate factors reducing the blood sugar also from other organs than the pancreas. Attention was in the first instance directed to the duodenum. The first to investigate such an effect of the intestinal mucosa was LOMBROSO 1924, who was able to induce, by means of intestinal extracts from both a normal and a pancreatectomised dog a fall in the blood sugar in another dog to below 80% of the initial value. In 1924 IVY and FISHER also isolated from the stomach and duodenal mucosa of the pig a substance reducing blood sugar, which had a beneficial effect on a diabetic dog. Particularly attempts have been made to extract from secretin a factor causing hypoglycaemia.

In 1929 ZUNZ and LA BARRE found in the following manner that secretin had an influence inducing hypoglycaemia: they performed a pancreatico-jugular anastomosis between two dogs, by connecting the pancreatic vein of one dog with the jugular vein of the other. On the injection of secretin into the saphenous vein of the donor, the blood sugar of the donor fell somewhat, although the main part of the insulin was transferred into the other dog. The fall of the recipient's blood sugar was both stronger and more rapid. The last observers came to the conclusion that secretin heightens the insulin secretion.

In 1930 STILL isolated from crude secretin the secretin proper, cholecystokinin and the factor producing hypoglycaemia. When applied in doses of 10 mg, it reduced within 2 hours the blood sugar of the diabetic dog from 330 mg% to 200 mg%, and for the normal dog from 110 mg% to 63 mg%. This agent cannot be insulin according to STILL, since it is not destroyed by pepsin and trypsin, nor does it necessarily stimulate insulin secretion, as the pancreatectomised dog responds to it. Purified secretin does not affect the blood sugar (STILL and SHPINER in 1930). In 1930 LA BARRE and STILL obtained identical results. They also checked ZUNZ's and LA BARRE's anastomosis experiments and found them correct. Since jugulo-jugular anastomosis only brings about a slight

fall in the blood sugar, they concluded that the secretin effect is due to 2 factors at least:

1. Crude secretin per se reduces blood sugar.
2. It also increases the insulin secretion.

In 1934 La Barre and Ledrut obtained with »incretin» prepared by them from secretin the same effect in pancreatico-jugular anastomosis as was achieved with secretin. 30 mg incretin per os per kg of body weight in 24 hours sufficed to keep a pancreatectomised dog alive for several months.

In 1947 Bocchiotti, Bellotti and Langlade isolated from the duodenum of birds an agent producing hypoglycaemia, which was much more potent than Ledrut and La Barre's incretin. 2—3 mg per kg of body weight caused in the dog a strong hypoglycaemia within 1—2 hours. It also practically eliminated glycosuria in the diabetic dog.

Yet several authorities absolutely deny that secretin and duodenal extract in general have an effect provoking hypoglycaemia. Fujimoto 1918 could not observe any change in the blood sugar after secretin administration, nor could Mellanby in 1928. Furthermore, Best, Jephcott and Scott in 1932 were unable to extract the factor producing hypoglycaemia from any extrahepatic tissues, and they were of the opinion that all positive results were due to technical errors.

Attempts have also been made to prove that hydrochloric acid from the duodenum decreases the amount of blood sugar. Loew, Gray and Ivy in 1939—1940 were unable to observe any such effect, nor could they find that its intra-venous application improved the utilisation of sugar. In their opinion, all duodenal extracts were absolutely ineffective as well.

Glucose tolerance curve.

It has been necessary to discuss all factors influencing the carbohydrate metabolism in order to form a basis for the understanding of the so-called auto-regulation of the blood sugar. This very question was for the first time clearly described and discussed by POLLAK in 1923.»The blood sugar elevation illustrates in all probability the adequate stimulus regulating the amount of the blood sugar supply from the liver.» According to him, its site of influence was to be found in the liver, the central nervous system or the endocrine glands. We have seen that it is to be found both in the liver, the peripheral tissues and the endocrine glands, as well as to some extent in the central nervous system. The usual blood sugar curve is therefore produced — the normal endocrine balance prevailing — in such a way that hyperglycaemia directly effects on the one hand, the following processes:

1. The sugar secretion of the liver into the blood diminishes or it begins to absorb sugar. This is followed by:
2. Acceleration of the glycogen formation in the liver.
3. Diffusion into the extracellular fluid, followed by
4. Acceleration of the glycogen formation in the tissues
5. Improvement of the muscular utilisation,

and finally of course

6. The excretion of sugar into the urine.

On the other hand, hyperglycaemia affects the glands of inner secretion, which in their turn affect the above processes.

1. The secretion of insulin increases and insulin is activated, probably due to a diminished pituitary secretion. This has the same effect on the liver as hyperglycaemia, i.e. its glucose secretion

diminishes. It is impossible to say whether or not it increases the glycogen formation in the liver over and above that which happens through hyperglycaemia and the basal insulin secretion. Injected insulin does not in fact produce this effect. The increase of the insulin in the peripheral tissues and its activation evidently increases the glycogen formation. It is also difficult to say whether additional insulin increases the utilisation (see p. 15).

2. The pituitary secretion is probably diminished. This results in a decrease of the blood sugar. Glyconeogenesis probably decreases, which partly occurs perhaps through the adrenal cortex and the thyroid.

3. The secretion of epinephrine decreases, which retards the breaking-up of glycogen in the liver, and possible the glyconeogenesis diminishes.

4. Nothing is known for certain of the reactions of the adrenal cortex and the thyroid gland to hyperglycaemia, but as already said, changes in the pituitary secretion way perhaps also affect them.

The above-mentioned factors affecting the glucose tolerance curve — apart from the hypothetical duodenal hormone — concern both the peroral and the intravenous glucose administration, since they all exert their influence through the rise of the blood sugar. Yet, there is at least the difference between enteral and peroral administration, that the sugar applied parenterally reaches the general blood circulation without even having passed through the liver. Another difference is that on the dose usually administered in the intravenous glucose tolerance test (0.5—1—1.5 gr/kg), the blood sugar rises to a much higher level than in the peroral tolerance test. It follows that the blood sugar concentration in liver capillaries probably rises in the intravenous tolerance test to an equal or even higher (0.2—0.5%) extent than in peroral tolerance test. Thus, it may be assumed that the effect of intra-venous tolerance test on the homeostatic mechanism of the liver is as strong as that of the peroral tolerance test, and that intravenous tolerance test has as great influence on the endocrine glands as the peroral one. It is therefore natural that intravenous tolerance tests are frequently recurred to, instead of the peroral method, for a determination of the glucose tolerance.

Moreover, intravenous tests have the advantage that the moment when the sugar passes into the circulation of the blood is exactly known. Therefore the intravenous tolerance test is more accurate than the peroral one in the estimation of the tolerance. As a rule, glucose is rapidly injected in a few minutes, without attempting to imitate the velocity of intestinal absorption, nor is it necessary according to the prevailing opinion. A smaller dose of glucose can also be used. »It is possible to evoke all the characteristic reactions that attend ingestion of glucose by rapid injection of the sugar, with less inconvenience and discomfort to the subject. A smaller quantity of glucose is required for this purpose by the intra-venous than by the oral route, since all the sugar that is given becomes immediately available.» (PETERS and VAN SLYKE in 1946).

The intravenous glucose tolerance curve.

The intravenous tolerance test is usually performed, as already said, by injecting glucose into the vein within a short time. Several observers administer the same dose to all subjects, irrespective of age and body weight. Thus JÖRGENSEN and PLUM in 1923 injected 20 g, TÖRNING 5, 10 and 20 g in 1932, LOZNER, WINKLER and TAYLOR 25 g, SOSKIN 25 g in 1944, FAIRLEY 50 g in 1936, ROSS 10 g to children in 1936. Others use certain doses per kilo of body weight: CRAWFORD 0.5 g/kg in 1938 and 1939, LENNOX 0.33 g/kg in 1927, TISDALL, DRAKE and BROWN 1 g/pound in 1925.

Immediately after the injection the blood sugar concentration is at its highest. The value obtained largely depends on how soon after the injection the test has been performed, as well as on the rate of injection. Immediately after a rapid injection the concentration is higher than after a slow injection. If on the other hand the first specimen is taken a few minutes after the conclusion of the test, an inverted effect may be obtained (JÖRGENSEN in 1926—27), probably because the high blood sugar following a very rapid injection induces a more rapid diffusion into the tissues.

The abrupt fall of the blood sugar curve after the injection seems initially to be of a similar form under any circumstances and in different individuals (first stage). Only after the blood sugar has

fallen to about 200 mg%, individual and conditional variations begin to appear (second stage) (JÖRGENSEN and PLUM in 1923, JÖRGENSEN in 1927). According to JÖRGENSEN, the fall in blood sugar while the first stage is in progress is due to the physical distribution of glucose between blood and tissues. It is only during the second stage that the metabolic factors begin to affect the form of the curve to a greater extent. This is also supported by the fact that the first stage is fairly normal in diabetics (JÖRGENSEN).

Hyperglycaemia induced by the intravenous tolerance test is generally followed by a hypoglycaemic stage, the blood sugar falling below the fasting level. This is evidently due to overcompensation caused by those factors, under the effect of which the blood sugar falls. Attempts have been made to explain this consecutive hypoglycaemia, as well as the Staub-effect, with the help of an increased insulin production during hyperglycaemia or by insulin kinase possibly generated in the course of it. Since however consecutive hypoglycaemia also appears in test animals whose pancreas has been replaced by a constant insulin infusion (SOSKIN and ALLWEISS in 1934), it is evident that consecutive hypoglycaemia is at least not solely due to an increased production of insulin.

For comparison of intravenous blood sugar curves the following characteristics of the tolerance curves have been taken into account:

1. Maximal elevation of the curve at a certain time after the injection (HIMSWORTH in 1934, SOSKIN in 1944). It is affected by the rate of injection, but also by technical errors particularly the time of withdrawal of the sample. The possibility of error is greater the sooner the specimen is taken after the injection.

2. The time within which the blood sugar attains its initial value e.g. JÖRGENSEN or the time which elapses until it falls to 0.1%, which especially CRAWFORD in 1938—39 recommends for children, since their fasting values vary much more than in adults.

When administering 0.33—0.5 g/kg, the adult curve falls to 0.1% as a rule in 60 to 90 minutes (LENNOX, SOSKIN). If the sugar dose is increased, the time of the fall is prolonged, however, not nearly in the same proportion as the extent of the dose. This is in fact fully comprehensible: the higher the blood sugar, the greater

the capacity of the normal organism to eliminate it. In FAIRLEY's experiments in 1936—37 where 5 g/kg were given, the average of 10 curves fell to below 0.1% in 90 minutes.

The glucose tolerance is higher in children than in adult life and it seems to be inversely proportional to their body weight. 20 g glucose produced a similar curve in adults and in children weighing 30—45 kg (JÖRGENSEN and PLUM). In CRAWFORDS experiments, where 0.5 g/kg body weight were given, the curves fell to 0.1% in subjects of different age within the following time:

0—2 years	.....	30—45 minutes
2—4 »	.....	45—60 »
4—10 »	.....	45—75 »
over 10 »	.....	60—75 »

JÖRGENSEN has used in the comparison of intravenous curves a so-called loading-figure, which is a projection of the intravenous curve on to the fasting line in mm plus the surface formed by the curve itself and its projection in cm^2 (area). Several investigators are however of the opinion that JÖRGENSEN's method of calculation is much too artificial, e.g. CRAWFORD in 1939.

The peroral glucose tolerance curve.

This is affected by a much greater variety of factors than the intravenous curve, whose form is largely determined by metabolic factors. These other factors being mainly of the kind that their influence on either curve cannot be determined, it is often difficult to estimate the sugar metabolism on the basis of a peroral tolerance curve. Such factors of unknown importance are for instance the emptying rate of the stomach and absorption velocity in the small intestine, as well as possible intestinal fermentation. The rate at which the stomach is emptied depends, as well known, to a great extent on nervous factors, both directly and since stimulation of the autonomic nervous system affects gastric secretion. Thus, even a slight irritation can increase, through vagal stimulation, the rate of emptying and therefore also the velocity of absorption.

As mentioned on page 17, vagal stimulation probably heightens insulin secretion, which may therefore partly reverse the previous effect.

When comparing peroral tolerance tests to each other, some authorities use the maximal height as a basis, for instance SOISALO in 1930, SVENSGAARD in 1931, BADENOCH and MORRIS, or the area, as HIMSWORTH and JÖRGENSEN, or the duration of hyperglycaemia (SOISALO). This is a relatively unreliable standard if the curve is lower then normal, when comparatively insignificant inhibitory factors, such as restlessness of the child, may provoke new, largely similar hyperglycaemic fluctuations as those induced by the proper glucose tolerance test.

50—100 g or 1—2 g/kg glucose per os generally induces in adults a maximum 200 mg% hyperglycaemia which reaches its peak within half an hour to 1 hour and disappears in 2 hours to 2 and a half. (SOISALO). Hyperglycaemia persisting for more than 3 hours must as a rule be considered pathological (SVENSGAARD in 1931). The increase of the dose does not greatly intensify hyperglycaemia, evidently partly because of the circumstance that the velocity of absorption is relatively independent of the dose, partly because, as already mentioned in connection with intra-venous tolerance test, the higher the blood sugar, the greater the capacity of the organism to eliminate it. Peroral, as well as intravenous glucose tolerance test is generally followed by consecutive hypoglycaemia, up to 50 mg% below the original value, not later than in 3—4 hours (SVENSGAARD). Sometimes an even stronger hypoglycaemic reaction is met with: MEULENGRACHT in 1930, 0.037 % and 0.011 %. In SOISALO's 100 tolerance tests consecutive hypoglycaemia was manifested in 84 cases already within 2 hours to 2 and a half.

Reports on peroral glucose tolerance tests in children are exceedingly contradictory. GOETZKY's maximal values in 1925 vary approximately within the same limits as in adults, 150—200 mg%, but he administered relatively large doses, 2—3 g/kg. HERLITZ in 1928 gave 1.3 g/kg to children from one and a half to 10 months old. He obtained a maximum average of 123 mg% in 30 min. to 1 hour. When administering much heavier doses of glucose (1—7 g/kg), he obtained higher maximal values (135—242 mg%).

BADENOCH and MORRIS, by giving 1 g/kg, obtained the following averages for maximal values in different age groups:

0—4	years	119 mg%
4—8	years	125 mg%
8—13	»	145 mg%

In the experiments of SPENCE in 1920—21, the tolerance was also inversely proportional to the body weight in children under three years when 2 g/kg was administered.

SVENSGAARD was unable to find any such correlation between age and maximal value of the peroral tolerance curve. On the contrary, her experiments where 2 g/kg were administered after a fast of 6 hours, yielded a maximum average of 172 mg% in a infant 1 year old, and the peak of the maximi fluctuated as much as from 109 to 250 mg %. In children of 1 to 3 years of age the maximal average was 155 mg % and variations between 122 and 201 mg%. SVENSGAARD drew the conclusion that children over 1 year old have a similar peroral sugar tolerance curve as adults. An increase of the dose above 2 g did not anymore intensify hyperglycaemia.

According to FRIES and KOHN, children from 3 to 13 years of age have the same tolerance if doses under 2 g/kg are administered. If doses surpassing 2 g are given, the tolerance becomes inversely proportional to the body weight.

Effect of diet on the glucose tolerance curve.

As already described on page 19, the sugar tolerance is in direct proportion to the carbohydrate amount contained in the diet prior to the test. This has been attributed to the following phenomena occurring in the course of the carbohydrate diet:

1. Insulin production increases or
2. Insulin is activated or
3. Pituitary secretion is reduced, which is surmised to have the same effect as the activation of insulin.

According to HIMSWORTH, diet does not consecutively affect the insulin production, therefore with regard to hormonal factors, only the two latter possibilities could be taken into account, i.e.

the activator and inhibitor theories, perhaps even both, since hypophysectomy reduced the difference in glucose tolerance on a diet rich in carbohydrate and one deficient in them, without however entirely eliminating this difference.

4. Glyconeogenesis is reduced, which is at least partly due to the preceding. This is, according to SOSKIN, that final factor to which the effect of diet on the sugar curve is due. »Both starvation and fat feeding are alike in that no preformed carbohydrate is being received by the body, so that the liver must make all the necessary carbohydrate from its own resources. This represents a high degree of activity on the part of the liver. The deceleration of sugar output by the liver when sugar is administered requires a longer time when the liver is working at top speed than when it is working at half or quarter-speed.»

According to SOSKIN and MIRSKY, in 1936 the sugar requirement of the tissues of dogs fasted more than 20 days before evisceration is normal, and therefore the poor tolerance on a diet deficient in carbohydrates is not at all due to peripheral factors. This is contested by the circumstance that after a diet poor in carbohydrates, sugar does not appreciably increase the respiratory quotient, its specific dynamic action is weak. E.g. BERGMAN and DRURY in 1937 also came to the conclusion that starvation impairs utilisation. They injected glucose into eviscerated rabbits at rates so regulated, that the blood sugar was maintained at a constant, normal concentration. Rabbits which had been fed up to the time of evisceration, utilised the glucose more rapidly than did rabbits which had been fasted for 4—6 days before evisceration.

The lowered tolerance to glucose after starvation has been explained, particularly by earlier authorities, as due to the breaking-up of glycogen, impairing the oxidation of sugar (»sugar is oxidised in the flame of glycogen»). This would also explain the diminished tolerance after heavy exercise, which uses up the glycogen reserves (STAUB in 1922, MCCLELLAN and WARDLOW in 1932, MILLS in 1938). This opinion is not perhaps fully contradictory to SOSKIN's theory, according to which pituitary secretion and therefore also glyconeogenesis are strong after heavy exercise, insulin is inactive and consequently perhaps the oxidation of sugar retarded. (See RUSSELL p. 22).

According to what has been said above, a diet deficient in carbohydrates and starvation exert such an effect on the peroral glucose tolerance curve, that it rises above the normal level and hyperglycaemia has a longer duration than normally, consequently it resembles a diabetic curve. Notwithstanding, that even a relative fasting retards the rate of emptying of the stomach (HENSCHEL and MACSURGEON) and the rate of absorption (DONHOFFER in 1942). Thus a diet deficient in carbohydrates of 2 days' duration raised according to KAGEURA the maximum of the peroral glucose tolerance curve by 60—70%. In Mc CLELLAN's and WARDLAW's experiments in 1932 a transition from a daily carbohydrate amount of 350—400 g to a 25 g dose induced a rise of the maximum from 0.130% up to 0.210%.

In children a diet deficient in carbohydrates lowers the glucose tolerance even more than in adults. In LIVINGSTON's and BRIDGE's experiments in 1942 with administrations of 3 g/kg to young infants, the maximum rose from 0.150—0.170% on a diet rich in carbohydrates to 0.200—0.240% after a low carbohydrate diet.

According to SOISALO, in 1930, the variations in the ordinary free diet have no effect on the peroral sugar tolerance curve.

Restricted carbohydrate intake and starvation affect the intravenous glucose tolerance curve in the same way as the peroral one, (e.g. HIMSWORTH in 1934) as is only natural. Therefore CRAWFORD's report in 1939 of a fully converse result is of greatest interest and very difficult ta explain. She kept healthy children for 3 weeks (4 experiments) on a carbohydrate starvation. The subsequent intravenous glucose tolerance curve was fully normal and fairly identical with the previous curve, although the children developed severe ketonuria.

The Staub effect.

It has already been mentioned that if glucose is administered in consecutive doses two or more times, the second and the following ones always induce a smaller rise of the blood sugar. This phenomenon was discovered by BANG in 1913. HAMMAN and HIRSCHMAN in 1919, gave 100 g glucose at intervals of one and a half to three hours. STAUB in 1921 administered to adults several times and at small intervals a constant dose of 20 g glucose per os, while hyperglycaemia produced by the preceding dose was still prevailing. Subsequent administrations failed to induce a new rise, on the contrary, the blood sugar diminished the whole time. He suggested that a »Bahnung» occurred in the formation of glycogen and oxidation of sugar. TRAUGOTT in 1922 obtained a similar effect in healthy individuals by giving at first 20 g and after one hour 50—100 g glucose. He explained it as due to the better capacity of the liver to absorb sugar during the second dose. FOSTER obtained the same phenomenon in 1923 and surmised that ingestion of glucose or hyperglycaemia stimulated the inner secretion of the pancreas so, that during the second test there was an excess of insulin in the blood.

The Staub effect has been studied later by several investigators, both by peroral and intravenous glucose tolerance tests. Several have obtained it in healthy adults by both methods, although there were some exceptions, MALMROS in 1928 as well as BARRENSCHEEN and EISLER in 1926 were unable to observe it. EXTON and ROSE in 1934 administered the second dose 30 minutes after the first one, while hyperglycaemia was still prevailing, and made determinations of the blood sugar before the test, before giving the second dose and 30 minutes after the second dose. He considered

the response still normal if the blood sugar was 10 mg% higher in the third than in the second determination. GOULD, ALTSCHULER and MELLEN in 1937 and MATHEWS, MAGATH and BERKSON in 1939 made their experiments mainly in a similar way.

The Staub effect in children has been studied by GOETZKY in 1925, who obtained it in an infant 4 months old by administering to it about 2.7 g/kg at intervals of one hour and a half, as well as by SCHÖNFELD, who obtained an inverted Staub effect in children — the second tolerance curve was higher than the first. SCHÖNFELD began his experiment after only 4 hours fasting, and explained his results by suggesting that the first dose was unable to compensate for the slower sugar metabolism induced by the longer fast, which exercised its effect on the second test. BOTT obtained in 1936 an inverted Staub effect in premature infants and an irregularly effect in children under 4 years age.

In intra-venous sugar tolerance tests the Staub effect has been obtained e.g. by HIMSWORTH in 1938 and SOSKIN in 1944. According to Himsworth, the Staub effect is clearly manifested if the test animal has been on a diet rich in carbohydrates, whereas on a low carbohydrate diet a very pronounced inverted Staub effect is obtained. Only if several tolerance tests are performed in sequence, the tolerance improves at last and the Staub effect manifests itself. SOSKIN performed his tests by administering 25 g glucose three times intra-venously at 2 hours' intervals (»triple tolerance test»). The Staub effect is then clearly manifested in healthy individuals, the peaks of the curves flattening out and the blood sugar falling to normal with growing rapidity.

ADLERSBERG and PORGES obtained results in 1926, which apparently contest all others. They administered initially 25—50 g glucose intravenously, and 60 minutes later 100 g by the mouth. This peroral glucose tolerance test produced gross hyperglycaemia, about 200 mg%, in spite of the preceding intravenous application. If, on the other hand, 25 g were first given by the mouth, the subsequent peroral administration of 100 g hardly provoked any hyperglycaemia.

As already said, the Staub effect was at first explained after the discovery of insulin by surmising that the first dose of glucose stimulated the insulin production, thus causing a surplus of it in

the blood during the second application. (Particularly ZUNZ and LA BARRE.) This theory of the origin of the Staub effect became improbable when SOSKIN, ALLWEISS and COHN in 1934 obtained an intra-venous Staub effect in a depancreatised dog, whose pancreas was compensated for by a constant infusion of insulin. Thus no extra insulin is needed for the occurrence of a Staub effect. SOSKIN and LEVINE explained the Staub effect on these grounds as a progressive depression in the activity (production of the glycotropic or anti-insulin factor) of the anterior pituitary gland as a result of repeated or prolonged exposure to hyperglycemic levels. Thus, after several successive doses of sugar the normal animal reaches that stage from which the hypophysectomized animal starts out». In hypopituitarism, according to them, an inverted Staub effect is generally obtained.

That same year, 1934, HIMSWORTH advanced his insulin kinase theory, and explained the Staub effect by suggesting that the hyperglycaemia caused by the first dose activates insulin, which therefore has a stronger effect during the second dose. Thus the difference between the two latter theories is that while HIMSWORTH supposed the Staub effect to be due to an activation of insulin, SOSKIN ascribed it to a decrease in the inhibition of the insulin effect.

In 1938 HIMSWORTH and SCOTT obtained their results described on page 23, and lent their support to SOSKIN's opinion, in so far as the variations in the sugar tolerance induced by the diet were surmised as largely due to the pituitary gland. Yet although hypophysectomy practically abolished the difference in sugar tolerance on a diet rich in carbohydrates or a diet deficient in them, — it *did not destroy the Staub effect.* Therefore, according to these experiments, the Staub effect cannot solely be due to a diminished pituitary secretion.

To summarise, the following theories have been advanced on the mechanism of origin of the Staub effect:

Hyperglycaemia

1. increases the insulin production, producing a surplus of it in the blood during the second dose,

2. activates insulin, which therefore exercises a stronger effect during the second dose,

3. decreases pituitary secretion, resulting in a decrease of the inhibition of insulin effect, which therefore has a stronger effect during the second dose.

According to SOSKIN et al., an increase of the insulin production is not needed for the appearance of the Staub effect. But since the insulin secretion evidently increases during hyperglycaemia, it is of course possible that this factor also has its share in the production of the Staub effect. SOSKIN maintains as well that the activation theory is also superfluous, although not inconsistent with the other theories: it can be easily surmised that in SOSKIN's experiments, where the pancreas was compensated by a constant insulin infusion, the Staub effect was manifested through the activation of insulin. SOSKIN did not, in fact, obtain the Staub effect in a hypophysectomised dog, but HIMSWORTH succeeded in producing it in such an animal. If we take this positive result of HIMSWORTH's as a starting point, we come to the conclusion that some other factor, beside an increased insulin production and diminished pituitary secretion, also plays a part in originating the Staub effect. This can be insulin kinase, or a diminished secretion of some other endocrine gland inducing hyperglycaemia — thyroid, adrenals, — or it can be some other factor, as yet unknown, productive of hypoglycaemia, such as the duodenal hormone already mentioned. An indication in this direction is perhaps given by the interesting work of ADLERSBERG and PORGES, where the intra-venous sugar dose did not lower the subsequent peroral tolerance, instead, this effect was attained by the peroral dose. This suggests that hyperglycaemia is not always essential for the manifestation of the Staub effect, other factors being perhaps the intestinal absorption of glucose or its passage through the liver.

Writer's Experiments.

Intravenous Two-dose Tolerance Test.

Intravenous double and triple tolerance tests were performed on healthy children, 27 tests in all. These children had all been in hospital for several weeks and were accustomed to injections and withdrawals of blood specimens, Prior to the test, all had been kept for at least one week on a fully normal diet. The standard diet was neglected, because according to SOISALO it is not important and because it is always very difficult to keep young children on a standard diet. The injection was made either into the jugular or the cubital vein; the duration of the injection was generally 2 to 3 minutes. The amount of glucose injected varied between 0.5 and 2 grams per kilogram of body weight, and it was usually given as a 10% solution, sometimes as a 20% solution, to avoid the administration of an excessive amount of fluid. The interval between the injections varied from 40 to 200 minutes. The test was performed in the morning at about 8 o'clock, after the night fast. If some quantity of the glucose solution, however insignificant, penetrated under the skin, the experiment was discontinued. The children were persuaded, successfully as a rule, to be fully at their ease before the test was applied, so that although nearly all of them cried under injection, they were quiet and contented after it while the blood specimen was withdrawn; during the second injection they usually behaved in the same way as previously.

The blood sugar was determined throughout by the HAGEDORN-JENSEN micromethod. The blood was withdrawn from the finger tip or the heel with Francke's needle, and each blood sugar level was determined as an average of 2 parallel tests. Both parallel

values have been included into the appended tables (1, 2, 3, 4). Below some detailed examples of such tolerance tests:

Fig. 1. Both curves rose to approximately the same level, about 250 mg% and fell to 100 mg% within the same time — 38 minutes and 37 minutes. Consecutive hypoglycaemia was considerably stronger after the second (85 mg%) than after the first (98 mg%) tolerance test. The interval between the injections was 60 minutes.

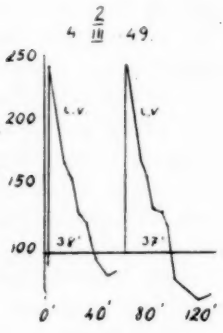


Fig. 1.

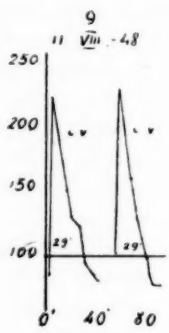


Fig. 2.

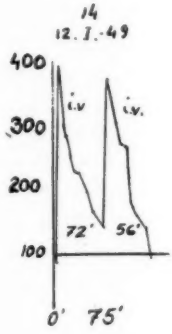


Fig. 3.

and the former curve was clearly ascending again at the time the second injection was applied.

Fig. 2. Both curves rose to the same level and descended within the same time to 100 mg%. The interval between injections was 40 minutes, and the former curve was still descending but already in the hypoglycaemic stage (last value 82 mg%) when the second injection was administered. Consecutive hypoglycaemia was somewhat stronger in the second test.

Fig. 3. The peak of the second curve was somewhat lower than the first and returned more rapidly. The second tolerance test was applied before the former curve had gone down to normal (last value 119 mg%).

Fig. 4. The interval between injections was much longer than ordinary, 190 minutes. The maximum of the second curve was higher than the first by 25 mg% and it took 5 minutes longer to fall. Consecutive hypoglycaemia was similar in both.

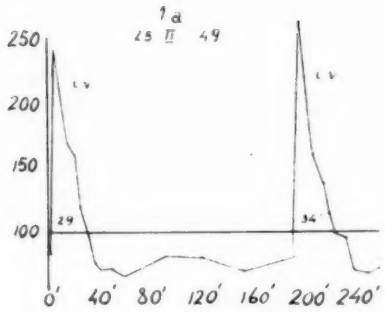


Fig. 4.

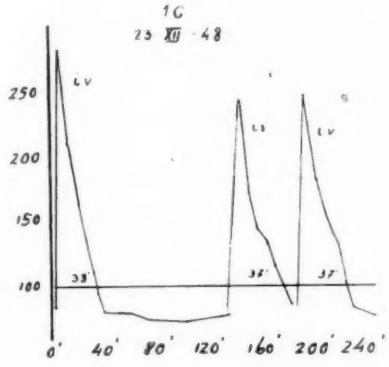


Fig. 5.

Fig. 5. Three successive tests were made, the second 150 minutes after the first, and the third 50 minutes after the second. The second and third had lower maximi, but both fell within a somewhat longer time (37 minutes) than in the first. Consecutive hypoglycaemia was practically identical in all three.

Fig. 6. 1.6 g/kg were administered three times, with a constant interval of 105 minutes. The second curve had a lower peak than the first, but the third was highest of all. The time of the fall became prolonged — 51 minutes, 69 minutes and 82 minutes respectively. Consecutive hypoglycaemia was 55 mg% in the first

test, but already not more than 70 mg% in the second and third tolerance test.

The rest of the experiments was fully consistent with those already described. The results are illustrated on Table I. In two cases the time required for the return to 0.1% in the second intravenous curve was unmistakably shortened (No. 14, N:o 1 b 16/9), but of them the third fall was already more prolonged than the

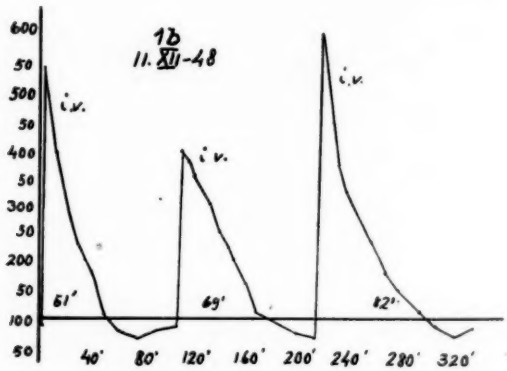


Fig. 6.

first in case No. 1 b. In the remaining 25 experiments the time required for the blood sugar to fall to 0.1 mg% was not shortened.

Fig. 7 shows the average curves for the 0.5 g dose. In the first the interval is from 40 to 60 minutes, in the following over 60 minutes. The time of the fall of the blood sugar to 0.1 was 35 and 35 minutes in the first, in the others 35 and 38 min. respectively. The peaks are practically speaking equally high in all the 4 curves.

The surface areas of the average curves have a ratio of 23: 22 and 22: 24.

It has been said already that the maximum height of an intravenous glucose tolerance curve is an uncertain standard for its assessment, particularly if the determination is made immediately after the injection, as in these experiments. By studying table I it becomes evident that the maximum of the second curve was

TABLE I

Intravenous two-dose tolerance test in healthy children and adults.

Date	Test person	Age (years)	Gluc./kg (g)	Interval (min.)	Fast. Val (mg %)	Max.[1] (min.)	Length[2] (min.)	Length I—Length II (min.)
11/ 9—48	1 a	1 4/12	0.5	40	I 90	237	34	
					II	247	35	— 1
16/ 9—48	1 b	1 4/12	0.5	40	I 97	269	38	
					II	269	30	8
					III	232	40	— 2 (I—III)
11/ 8—48	10	1	0.5	40	I 85	240	39	
					II	270	42	— 3
11/ 8—48	9	1	0.5	40	I 85	223	29	
					II	225	29	0
7/ 3—49	3	12	0.25	100	I 78	193	46	
					II	189	41	5
7/ 1—49	11	1 11/12	0.5	60	I 79	233	29	
					II	228	27	2
4/ 3—49	2	5	0.5	60	I 91	245	38	
					II	249	37	1
11/ 2—49	12	5	0.5	60	I 81	262	32	
					II	231	31	1
3/ 6—49	8	8	0.25	65	I 83	165	38	
					II	203	37	1
18/ 9—48	4	5	0.5	75	I 92	207	24	
					II	242	26	— 2
26/ 2—49	2	5	0.5	105	I 85	253	40	
					II	215	38	2
22/ 6—49	45	11	0.5	130	I	219	40	
					II	249	46	— 6
23/ 2—49	6	6	0.5	145	I 82	247	33	
					II	207	36	— 3
22/ 2—49	7	7	0.5	180	90	272	40	
					II	279	44	— 4
11/ 3—49	2	5	0.5	180	I 75	233	22	
					II	283	24	— 2
1/ 3—49	1 c	2	0.5	200	I 84	229	30	
					II	256	34	— 4
21/12—48	5	5	0.5	75	I 74	258	39	
					II	252	42	— 3
23/12—48	1 c	2	0.5	150	I 83	286	33	
					II	245	37	— 4
					III	248	37	— 4 (I—III)

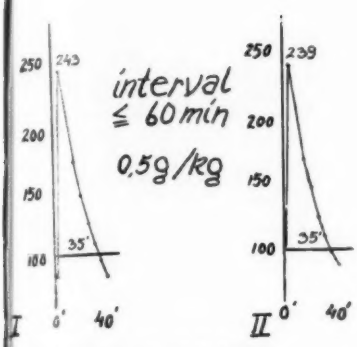
[1] 3 minutes after injection.

[2] Falling time of the blood sugar to 0.1 %.

TABLE I (cont.)

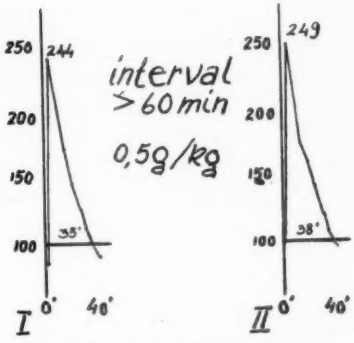
Date	Test person	Age (years)	Gluc./kg (g)	Interval (min.)	Fast. Val (mg %)	Max.[1] (mg %)	Length[2] (min.)	Length I– Length II (min.)
25/ 2—49	1 a	2	0.5	190	I 79	236	29	
					II	263	34	— 5
9/ 3—49	1 b	2	0.5	195	I 77	220	41	
					II	240	46	— 5
24/ 2—49	2	5	0.5	210	I 95	274	36	
					II	373	44	— 8
15/ 1—49	15	3 6/12	1	105	I 83	186	40	
					II	361	51	—11
					III	361	60	—20 (I—III)
12/ 1—49	14	2 3/12	1	75	I 85	379	72[3]	
					II	368	56[3]	16
18/ 1—49	1 c	2	1	70	I	493	44	
					II	470	39	5
					III	494	46	— 2
11/12—48	1 b	2	1.6	105	I 85	542	51	
					II	400	69	—18
					III	590	82	—31 (I—III)
7/12—48	1 a	2	2	110	I 82		65	
					II		72	— 7
9/12—48	13	1 6/12	2	110	I 90	660	61	
					II	660	77	—16
2/ 9—48	16	31	0.25	45	I 101	243	33	
					II	201	29	4
2/10—48	17	20	0.25	70	I 94	171	44	
					II	172	37	7
					III	165	34	10 (I—III)
15/ 6—49	22	20	0.3	75	I 105	227	58	
					II	240	48	10
8/ 6—49	18	21	0.3	80	I 97	244	54	
					II	192	34	20
13/ 6—49	20	24	0.3	95	I 107	238	46	
					II	230	37	9
8/ 6—49	21	21	0.3	80	I 106	216	77	
					II	230	45	22
17/ 6—49	9	19	0.3	190	I 103	234	54	
					II	214	70	—16
21/ 6—49	22	20	0.3	140	I	217	57	
					II	230	60	— 3

[1] 3 minutes after injection.
[2] Falling time of the blood sugar to 0.1 %.
[3] » » » » » » » 0.140 %.



Mean curves of 7 double tolerance tests.

Fig. 7.



Mean curves of 11 double tolerance tests.

only in 8 cases unmistakably lower than that of the first, but in all these cases the fall of the second curve was as slow or slower than in the first.

Thus the foregoing experiments failed to reveal in children a clear intra-venous Staub effect, with the exception of 1 experiment (14, 12/5—49 Fig. 3).

In Soskin's experiments, where the intravenous Staub effect was clearly manifested in adults, 25 g glucose were administered, i.e. about 0.3 g per kg body weight. Infants have a higher glucose tolerance, therefore 0.5—1.0 g per kg weight approximately correspond to Soskin's dose.

Himsworth has used for adults 1.0 g/kg, which probably corresponds to a somewhat larger dose in children (1.5—2.0 g/kg). The doses administered remained just within these limits, and one feels therefore that the non-appearance of the Staub effect is not due to the amount administered.

The interval between the injections can also hardly be the cause of the negative result. Soskin made his experiments with 2 hours' intervals. In my own experiments, the interval varied between 40 and 200 minutes, and the result was negative, apart from the exception already mentioned, irrespective of whether the blood sugar was or was not falling at the time the next tolerance test was instituted.

The blood sugar can react in the following ways at the beginning of the second i.v.-tolerance test:

1. It is going down and the last value is normal or subnormal.
2. Consecutive hypoglycaemia has passed off and the blood sugar shows a tendency to rise.
3. »Fluctuation» has passed off and the blood sugar been stabilised to normal.
4. Hyperglycaemia produced by the first is still prevailing.

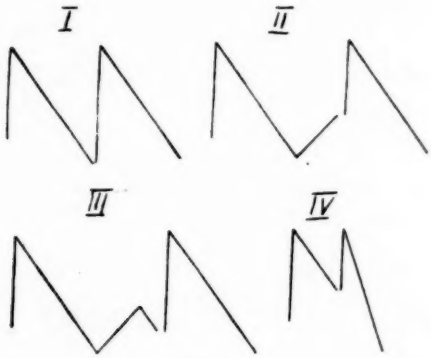


Fig. 8.

It is evident that different results can be obtained, according to the stage during which the second tolerance test is performed. By studying the figure on p. 50 one can observe that the liver absorbs sugar from the blood while hyperglycaemia is still prevailing. The blood sugar having fallen to normal, the liver remains passive for some time, and then begins to excrete glucose as before the performance of the test. The Houssay-dog's glucose tolerance curve being practically normal but that of a hepatectomised dog diabetic, this liver function occurs, partly at least, independently from the pancreas and the pituitary. If the second tolerance test is now performed while hyperglycaemia induced by the first is still prevailing, (possibility 4), the comparatively independent regulatory mechanism of the liver causes a slighter rise and a more rapid fall of this curve. Such a Staub effect is therefore partly produced by

the same mechanism which is responsible for the normal glucose tolerance curve and, in the light of SOSKIN's experiments, it seems unnecessary to assume that the pancreas and the pituitary gland have any share in it. Staub made his first experiments in this way, i.e. by administering glucose doses of 20 g at short intervals, and during the terminal tests the blood sugar decreased almost regularly. By shortening the intervals between the doses still more, a continous glucose administration is naturally achieved, in which the »Staub effect» is also manifested.

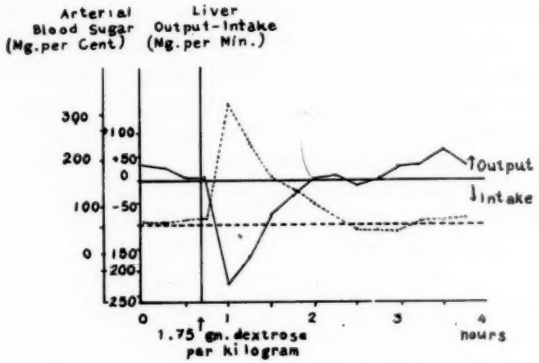
If the second i.v.-tolerance test is performed after the blood sugar has returned to normal (Possibilities 1—3), the appearance of the Staub effect must be due to some factor with an effect of longer duration than that of hyperglycaemia, or according to the theories either an increased insulin secretion, activation of insulin or the inhibited secretion of the glycotropic factor in the hypophysis.

Such a Staub effect is not, at least in intravenous tolerance tests affected by the liver still absorbing sugar from the blood at the beginning of the second test, as in the foregoing case, instead, the influence is due to the intensification of this capability in the liver and perhaps in the peripheral tissues, since, according to recent opinion, the first tolerance test affects some or all of the three factors mentioned.

In Possibility IV these factors are evidently doing their share, and in addition, the regulatory hepatic mechanism is functioning, which is sufficient to produce a normal blood sugar curve and consecutive hyperglycaemia. Therefore, if we wish to study the Staub effect as a separate phenomenon from the simple glucose tolerance curve, the second intravenous test must be performed after the subsidence of hyperglycaemia.

Thus, in the experiments under discussion, the acceleration of the blood sugar elimination did not persist until the following test, with the exception of that case (No. 14), where the second test was performed while hyperglycaemia induced by the first was still prevailing. This experiment does not therefore demonstrate any pure Staub effect.

It appears that if the interval between the tolerance tests is prolonged over 60 minutes, a slight negative Staub effect is obtained, the second curve lengthens. The difference is not great, 2



Direct demonstration of the homeostatic mechanism in the liver. The effect of dextrose administration upon the output and intake of sugar by the liver of an intact dog, calculated from bloodsugar values and thermostromuhr measurements of hepatic blood flow. The broken line represents arterial bloodsugar values; the heavier, continuous line represents output or intake of sugar by the liver in milligrams per minute. Note the immediate cessation of sugar output when sugar is administered and the large intake of sugar which follows. Throughout the second hour after sugar administration the liver neither retains nor excretes sugar. During this period the level of sugar in the arterial blood falls below its original control values and does not return to normal until after the liver has resumed its output. SOSKIN et al. 1938.

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to 8 minutes, but the tendency seems evident, since there is only one exception among 11 tests, and even that with a shortening of 2 minutes only (No. 2, 26/2). In all these experiments the blood sugar had already become stabilised at the beginning of the second tolerance test, and the hepatic sugar secretion was therefore evidently normal. It is difficult to make any deductions concerning the causes of this phenomenon — provided it proves constant in a more extensive experiments. But it may be assumed as due to the »pendulum swings» of that factor which produced the Staub effect, i.e., that the absence of this factor or counterregulatory factors cause at a certain stage an inverted effect, the same that appears in hypopituitarism and in carbohydrate starvation (SOSKIN, HIMSWORTH) and which may be interpreted as some kind of an exhaustion phenomenon of the blood sugar regulation. This interpretation presupposes however that the intra-venous Staub effect is

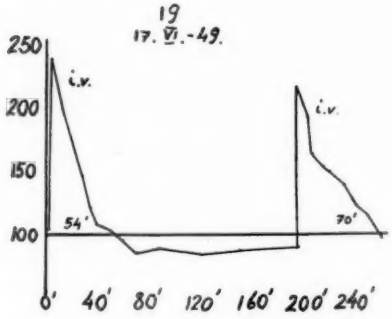


Fig. 9.

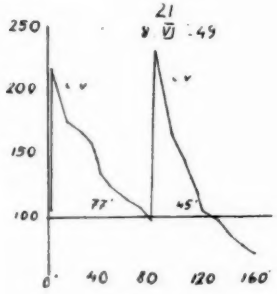


Fig. 10.

manifested at some suitable interval between the tolerance tests — the pendulum swings back only after having gone in the opposite direction.

It is also probable that if the intravenous Staub effect is so clearly manifested in adults, it cannot be fully absent in children. A slight tendency towards its manifestation is in fact evident in some of my own experiments and it is fully possible that it would have been somewhat more pronounced, had shorter intervals than 40 minutes have been used. A clear Staub effect would hardly have appeared even under these circumstances, since a shortening of the interval by some minutes would indicate already that the second tolerance test had been performed while hyperglycaemia still persisted.

From those 6 tests where a heavier dose (1.0—2.0 g/kg) was administered, in 5 experiments a negative Staub effect was obtained which was fairly pronounced. Particularly the triple tolerance test performed on No. 1 b in 11/12—42 greatly resembles the results obtained by SOSKIN in hypopituitarism by his triple tolerance test Fig. 6. It could be assumed also that an exhaustion of the blood sugar regulation was prevailing, in this caseresulting from overstimulation.

Seven adults were used as controls and subjected to a similar intra-venous double tolerance test, as described above for children. Figure 10 and Table I illustrate a clear manifestation of the Staubeffect fully reminiscent of SOSKIN's experiments, in all other experiments with the exception of the two (No. 19 and 22, Fig. 9) where the interval was very long, 190 and 140 minutes. The falling time in Fig. 19 was prolonged here from 54 to 70 minutes, i.e. the same phenomenon was present as in children, but greatly intensified.

In addition, both curves are fairly identical in that experiment (N:o 16) which shows a shorter intravenous tolerance curve than is usual in adults.

Peroral Two-dose Tolerance Test.

Twenty two two-dose tolerance tests were performed on children aged from 6 months to 9 years. The test was begun, as in the foregoing intravenous series, at 8 o'clock in the morning after the night fast, the children having been in the same way on a normal diet for at least one week. Glucose was administered as a 10% solution 2 g per kg weight. It was generally attempted to let the child drink the solution of its own will. If this appeared to take more than 2—3 minutes, it was introduced into the stomach by a rubber tube. Usually the child could be quietened down already by the beginning of the experiment.

In peroral two-dose tolerance tests performed at 2½ hours' intervals the Staub effect was generally obtained, but there were also 5 clearly inverted reactions. Of these experiments 9 were performed on identical triplets (1 a, 1 b and 1 c). Their Staub effect

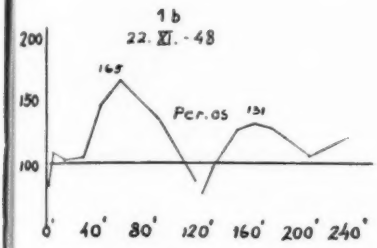


Fig. 11.

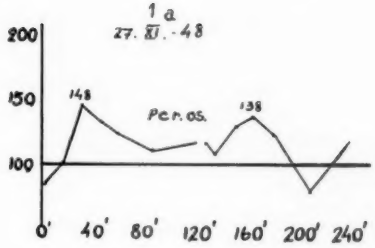


Fig. 12.

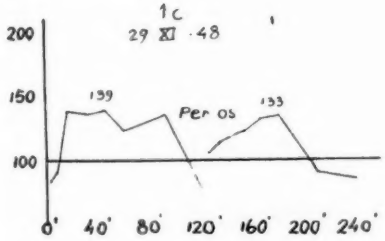


Fig. 13.

was fairly constantly positive, and in simultaneous tests fairly similar in all three of them. At first, in November 1948, the effect was manifested with moderate intensity. Fig. 11, 12, 13. Likewise in December, see table 2 (except on $^{14}/_{12}$ 1948, 1 b, where the Staub effect was weak. In this experiment, however, the dose was only 1 g/kg). The curves for March 1949 are more difficult to interpret. 1 b and 1 c had a flat first curve, 1 a, a fairly high one, but all three had a practically similar second curve: the peak was attained already in 5 minutes after the drinking of the solution, and succeeded by a steep descent. Although the curve subsequently mounted somewhat, it appears that a strong tendency to hypoglycaemia was prevailing during the second test. The amount of catalysation is difficult to assess, particularly in the last series. This is also the case with other double tolerance tests, where no more than summary results can be obtained by analysing the curves as above:

Fig. 14; both curves were low. The second attained its maximum 5 minutes after the drinking of the solution, and began to fall again. The variations are so small that an analysis appears most uncertain.

Fig. 15: both curves practically identical, fairly low. The Staub effect appears to be slightly negative.

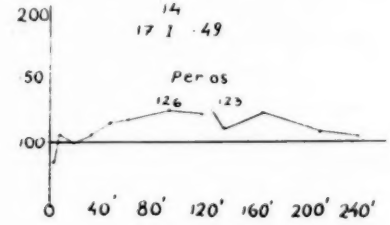


Fig. 14.

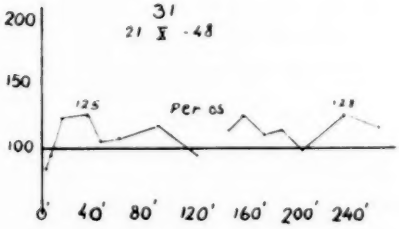


Fig. 15.

No. 6: second curve maximum in 5 minutes. Blood sugar beginning to fall in 30 minutes. Staub effect slightly negative.

No. 11: the first curve flat, the second higher, the Staub effect negative.

No. 23: the peak of the second curve was reached within 15 minutes, thereafter an abrupt fall. It appears as if the tendency of the blood sugar to fall were strong during the second test.

No. 24: two peaks in both curves. The peaks of the second curve were clearly higher. Staub effect negative.

No. 25: the second maximum slightly higher than the first, otherwise both curves fairly similar. Staub effect slightly negative.

TABLE II

Peroral two-dose tolerance test in healthy children.

Date	test person	Age (year)	Fast. Val.	Max. (mg %) Curve I	Max. (mg %) Curve II	Max. I — Max. II (mg %)
15/12—48	1 a[1]	1	87	169	128	41
22/11—48	1 b	1	80	165	131	34
15/12—48	1 b	1	87	164	134	30
27/10—48	30	7	76	149	128	21
27/11—48	1 a	1	93	148	138	10
11/ 1—49	25	1 4/12	76	141	149	— 8
6/ 4—49	1 a	1 5/12	78	139	122	17
29/11—49	1 c	1	83	139	133	6
9/ 4—49	26	2 1/12	93	137	103	34
30/11—48	27	1	93	131	105	26
29/11—48	29	3	96	129	101	28
11/ 2—49	6	6	81	129	134	— 5
13/ 1—49	28	1 5/12	62	128	126	2
17/ 1—49	14	2 3/12	87	126	123	3
21/10—48	31	9	84	125	128	— 3
12/ 4—49	23	6/12	95	124	121	3
8/ 4—49	24	1 3/12	70	113	135	—22
17/ 1—49	11	2	83	110	125	—15
11/ 4—49	1 b	1 5/12	78	109	116	— 7
7/ 4—49	1 c	1 5/12	73	101	112	—11
14/12—48	1 a	1	91	134	133	1 (1 g/kg)
14/12—48	1 b	1	85	144	138	6 (1 g/kg)

[1] 1 a, 1 b, 1 c are identical triplets.

No. 26: a pronounced strong Staub effect.

No. 27: the second curve quite flat. Staub effect clearly positive.

No. 28: the second tolerance test was done during slight hyperglycaemia. Falling tendency not particularly pronounced during the second test.

No. 29: the second curve did not rise at all. Strong Staub effect.

No. 30: the second tolerance test was performed while hyperglycaemia still persisted, therefore it does not illustrate the »pure« Staub effect. The second curve unmistakably lower than the first.

Strong consecutive hypoglycaemia did not appear in these experiments. The lowest value was that of No. 1 b, 11/4.—49, 67 mg%, 30 minutes after the beginning of the second tolerance test.

Table II shows that the Staub effect is at its strongest in those experiments where the maximal value of the first tolerance test is high, and appears slight in those where the first maximum is below 130 mg%.

It appears that there was no correlation between Staub effect and age in these experiments.

The form of a peroral glucose tolerance test is naturally always somewhat subject to hazards, since it is affected by several factors not easy to elucidate, as mentioned on page 32. One of them is the rate of emptying of the stomach, depending on many different conditions, such as nervous factors, hydrochloric acid concentration and rate of absorption, which can all be influenced by the same factors.

In double tolerance tests with an interval as long as 2 hours the factors affecting the emptying rate and rate of absorption referred to above may be entirely different during the second test than during the first. It is moreover quite possible that these factors are modified already during the first experiment. It can be assumed that a »Bahnung» phenomenon also occurrs in absorption. This appears probable, since, as already mentioned on p. 36, even a relative starvation affects the rate of emptying of the stomach and the rate of absorption. Thus it is possible that the first dose following the night fast is absorbed slower than the second. Therefore the peroral Staub effect need not always demonstrate only the metabolic change produced by the glucose administration, instead, it is an indicator of the sum total of the modifications of accidental factors and of the metabolic changes. We are not justified therefore in maintaining with absolute certainty that a positive peroral Staub effect signifies an accelerated elimination of the sugar, not that a negative one is a mark of no acceleration, as according to Schönfeld is the state of things in young infants.

Apart from being due to these factors, of an accidental nature, affecting the form of the peroral tolerance curve, it is difficult to interpret the Staub effect in children, since they frequently have

such a low peroral tolerance curve, that even should the first test catalyse the elimination of blood sugar during the second, this catalysation would not be particularly noticeable in the form of the second curve. In fact, high blood sugar is much more sensitive to factors inducing hypoglycaemia than low: much more insulin is needed to reduce the blood sugar from 100 to 90 than from 200 to 190 mg%. (HIMSWORTH).

Effect of Peroral Administration on the Intravenous Tolerance Curve

It seems to me that we shall perhaps be able to make a more accurate study of the effect of the peroral glucose tolerance test on the regulation of blood sugar by using an intravenous tolerance test instead of the second peroral administration, and by comparing the intravenous curve thus obtained to the intravenous tolerance curve previously obtained by the same individual. The »acceleration» of the intravenous curve would then serve as a standard for measuring the acceleration of the blood sugar elimination induced by the peroral administration. Such an experiment would have at least that advantage, that only the form of the curve the effect of which one intends to examine is subject to the influence of accidental factors due to the rate of absorption, whereas the »indicator», the intravenous tolerance curve, is determined only by those factors, which affect the blood sugar regulation.

Fig. 16 illustrates such an experiment. The previous intravenous glucose tolerance curve made on 16/I., fell to normal within 40 minutes. The subsequent peroral curve (2 g/kg) mounted to 138 mg%. The intravenous curve made on 17/I. fell to 0.1% in 34 minutes. The subsequent peroral tolerance test rose to 129 mg%. On 11/II. a peroral dose was given first and rose to 134 mg% by administering 2 g/kg, thereafter the intravenous test was made in the same way as on 16/I. and 17/I. by administering 0.5 g/kg. Now it fell to 0.1% in 20 minutes. Therefore, the shortening of the intravenous curve is 14—20 minutes, i.e. 41—50 %.

Fig. 17 represents an experiment where the peroral tolerance test was made first and the intravenous afterwards, and on

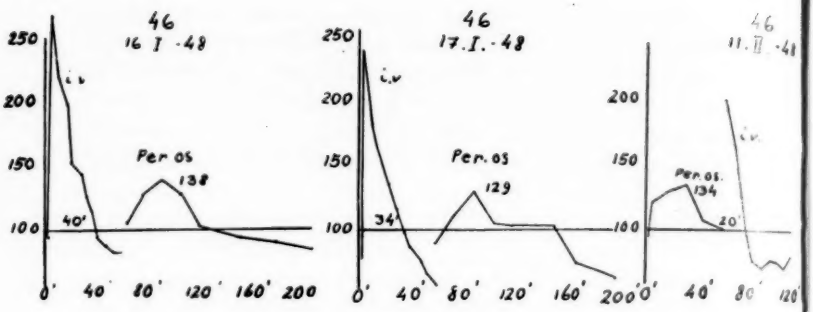


Fig. 16.

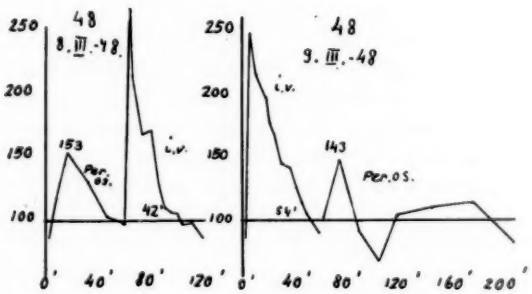


Fig. 17.

the following day both these tests in the reversed order. After the peroral dose the intravenous curve fell to 0.1% in 42 minutes, whereas the one made before it took 54 minutes. Shortening 12 minutes, i.e. 22%.

Fig. 18 illustrates the same experiment performed on on adult. The intravenous curve subsequent to the peroral tolerance test was 16 minutes, i.e. 30% shorter than the preceding intravenous curve.

In all these experiments the peroral curve made first was practically equally high as the one made after the intravenous test.

the differences being so small that they can be entirely ascribed to the variations of the peroral curve from day to day. The same possibility of variation naturally also applied to the intravenous curve, but not to such great extent. In the first instance, it is solely affected by metabolic factors, in the second one should bear in mind that since in intravenous application the sugar suddenly passes into the circulation, not even a considerably accelerated metabolism is capable of shortening the falling time to 0 minutes.

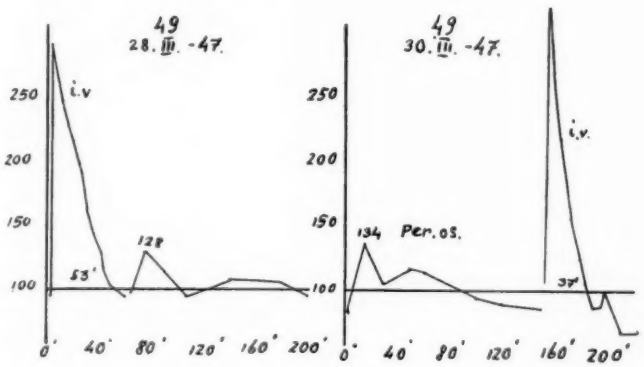


Fig. 18.

It may therefore be possible that while a maximal acceleration of the sugar metabolism is prevailing, the peroral tolerance curve does not mount at all, but for the fall of the intravenous curve some minimal time is still necessary. Consequently, a shortening of the intravenous curve for instance from 53 to 37 minutes, i.e. fairly close to the smallest possible interval, probably marks a much greater metabolic change than the descension of the peroral tolerance curve from 134 to 128 mg%.

The foregoing experiments seem to indicate therefore that the peroral glucose dose has shortened the following intravenous tolerance curve, but that the intravenous test has not greatly affected the following peroral test — i.e. the same observation as made by ADLERSBERG and PORGES.

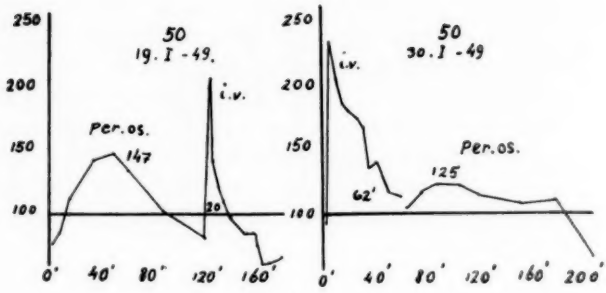


Fig. 19.

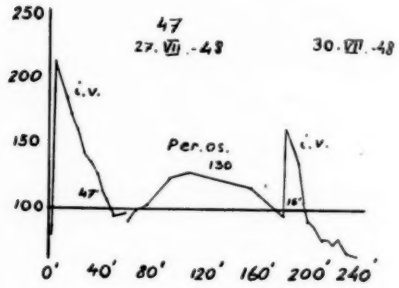


Fig. 20.

Results were different in the following test (Fig. 19) where the preceding peroral administration accelerated the intravenous curve from 62 to 20 minutes, but the preceding intravenous curve also reduced the maximum of the subsequent peroral curve from 147 to 125 mg%. In this case however the peroral dose following on the intravenous administration was given before the intravenous curve had flattened out to normal, i.e. the liver was still absorbing sugar from the blood at the beginning of the peroral tolerance test, thus contributing to its fall.

In Fig. 20 the intravenous curve made on 27/VII. fell in 47 minutes, a similar one made after the peroral test on the 30/VII. took 16 minutes. Shortening 29 minutes, i.e. 66%.

Intravenous-peroral-intravenous tests.

Since it appears that the intravenous glucose administration in children does not greatly affect a following similar test, the experiment reported above could perhaps be simplified so, that all three tests — intravenous, peroral and intravenous, are made successively. If the second intravenous tolerance curve now proves shorter than the first, this shortening is due in great probability to the intermediate peroral tolerance test.

Figures (21—32) illustrate such tests, 30 in all, which were performed on healthy children. The intravenous dose was 0.5 g both times and the peroral 2 g per kg weight. The interval between the first intravenous and the peroral tolerance test was 60—90 minutes, the interval between this latter and the second intravenous test was 120—180 minutes, usually 120 minutes. The rate of the blood sugar elimination was determined 1) through the shortening of the falling time to 0.1 % in minutes, 2) by calculating the percentage of this shortening from the falling time of the first curve.

In cases where the second intravenous curve or both of them did not fall to 0.1% while the experiment was in progress, they were compared to each other by using the falling time to some other blood sugar value (0.110—0.140%).

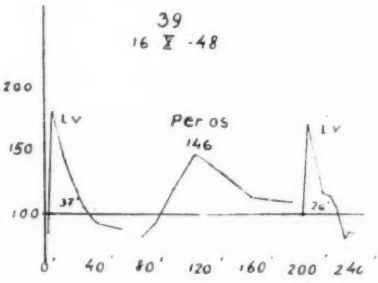


Fig. 21.

In Fig. 21 the falling time of the first intravenous curve was 37 minutes, the subsequent peroral curve rising to 146 mg%. The second intravenous test was made while a slight hyperglycaemia (110 mg%) was still persisting. The falling time of the curve was 26

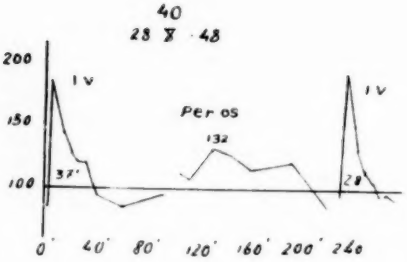


Fig. 22.

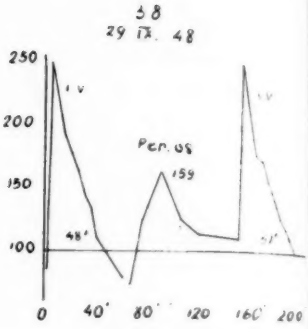


Fig. 23.

minutes. The shortening is therefore 11 minutes, i.e. 30%. Consecutive hypoglycaemia was 10 mg% stronger during the second than during the first intravenous tolerance test.

Fig. 22: the first curve fell in 37 minutes, the second in 28 minutes. The peak of the peroral curve was 132 mg%. The shortening was 9 minutes, i.e. 24%.

Fig. 23. The first intravenous curve fell in 48, the second in 51 minutes. The peak of the peroral curve was 159 mg%. Although the peroral curve was still descending when the second intravenous tolerance was applied, there was no shortening in the second intravenous curve.

Fig. 24. The first intravenous curve fell in 30 minutes. Peroral curve maximum was 164 mg%. On the administration of the second intravenous dose, it revealed again an ascending tendency,

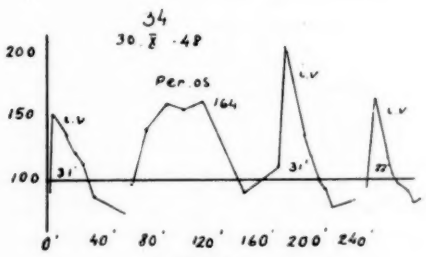


Fig. 24.

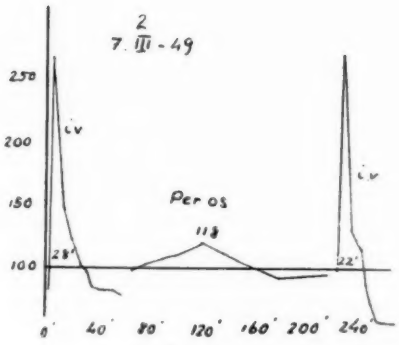


Fig. 25.

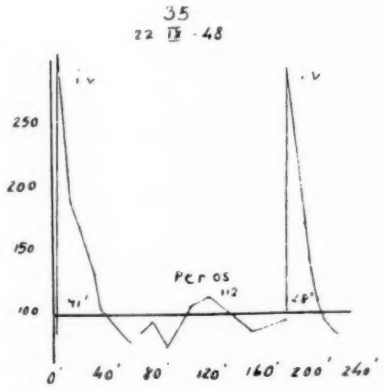


Fig. 26.

which probably induced, at least partly, the fall of the second intravenous curve within the identical time (31 minutes) as the first. Instead, the third intravenous curve which was made 60 minutes after the second, fell in 22 minutes.

Fig. 25. The first curve fell within 28, the second within 22 minutes. Peroral curve maximum 118 mg%. Acceleration was only 6 minutes, i.e. 21 %, but it is noteworthy that the second intravenous curve was so short that even a strong factor

accelerating metabolism could hardly shorten it anymore. It is probably to be interpreted as a mark of strong catalysation that consecutive hypoglycaemia was exceedingly pronounced in the second intravenous tolerance test — the blood sugar fell to 56 mg%.

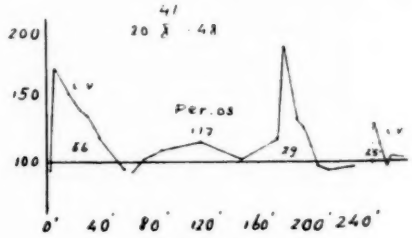


Fig. 27.

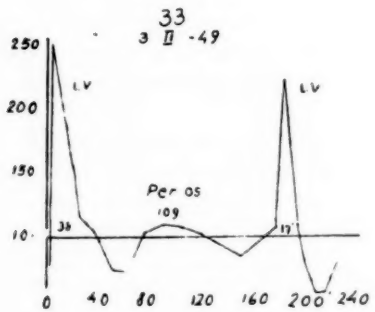


Fig. 28.

Fig. 26. The first curve fell within 41, the second within 28 minutes. Peroral curve maximum 112 mg%. Shortening 13 minutes, i.e. 32%.

In Fig. 27 the falling time of the first intravenous curve was 56 minutes. The peak of the peroral curve rose at first to 117 mg% only, but it was ascending again at the time the second intravenous dose was administered. Nevertheless, the falling time of the latter was only 29 minutes, acceleration being therefore 27 minutes, i.e. 48 %. The third intravenous tolerance curve made 60 minutes later had a slightly more rapid fall, i.e. in 25 minutes.

Fig. 28 strongly resembles the foregoing. The falling time of the first intravenous curve was 38 minutes, the peroral curve maximum only 109 mg%, and the curve was rising again when the second intravenous dose was administered which fell in 17 minutes. Nevertheless, the acceleration was 21 minutes, i.e. 55%. The strong catalysation is further revealed by the fact that consecutive hypoglycaemia was exceedingly pronounced during the second tolerance test — the blood sugar fell to 53 mg%.

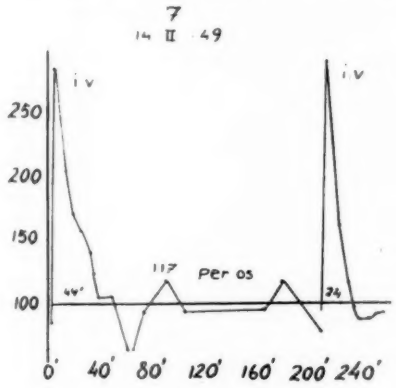


Fig. 29.

Fig. 29. The first intravenous curve fell in 44, the second in 24 minutes. Peroral curve maximum was 117 mg%. Shortening 20 minutes, i.e. 45%. Two days later (Fig. 30) the same child had a first intravenous curve of 45 minutes and the second 40 minutes duration, with a peroral curve maximum of 178 mg%. Shortening only 5 minutes, i.e. 11%.

Fig. 31. The peroral dose was administered before the blood sugar had gone down to normal, which possibly induced, at least partly, the failure of the peroral curve to rise at all. The first intravenous curve fell to 140 mg% in 39, the second in 22 minutes, acceleration being 17 minutes, i.e. 44%. Consecutive hypoglycaemia was exceedingly strong in the second intravenous test — the blood sugar fell to 59 mg%. Later on (Fig. 32) the same child had a fall of the first intravenous curve in 31 and of the second in

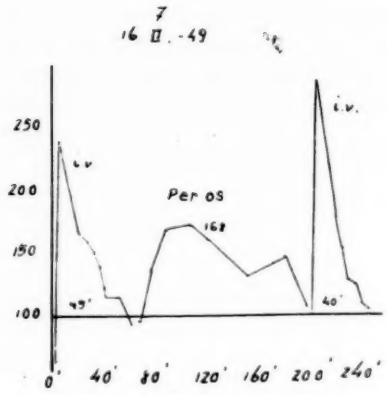


Fig. 30.

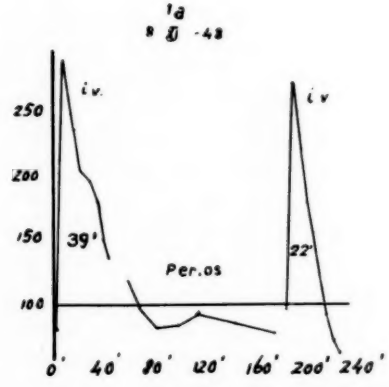


Fig. 31.

20 minutes. The peroral curve failed to rise again. Shortening 11 minutes, i.e. 36%.

It would take too much space to report all the 30 experiments in the same way as described above. Table (III) reveals that the peroral glucose tolerance test frequently catalysed the following intravenous curve. This occurred also in those cases where the peroral test did not induce any rise of the blood sugar worth men-

TABLE III

intravenous — per os — intravenous test in healthy children and adults.

Date	Test person	Age (year)	I i.v. Length	Per os		II i.v. Length	Katal. %
				max.	Area[4]		
				mg %			
21/ 2—48	1 b	1 9/12	37	121	5	22	41
8/11—48	1 a	1 5/12	39[1]	91	0	22	44
23/11—48	1 c	1 6/12	43	132	14	36	16
24/11—48	1 b	1 6/12	50	182	25	46	8
1/12—48	1 c	1 6/12	36	131	8	25	31
2/ 2—49	1 b	1 8/12	38	136	11	30	21
19/ 2—49	1 c	1 8/12	35	110	1	28	20
18/ 2—49	1 a	1 8/12	31	103	0	20	36
16/ 2—49	7	1 9/12	45[3]	168	56	40[3]	11
14/ 2—49	7	1 9/12	44	117	4	24	45
31/ 1—49	32	9	30	134	20	28	7
4/ 2—49	32	9	46	138	21	46	0
7/ 3—49	2	4 8/12	28	118	9	22	21
3/ 2—49	33	2 8/12	38	109	3	17	55
8/ 2—49	12	6	38	156	37	33	13
10/ 2—49	12	6	35	119	14	25	29
7/ 2—49	6	7	42[1]	163	63	49[1]	—17
17/ 2—49	6	7	32	124	8	27	16
28/ 1—49	15	2 7/12	25	92	0	18	28
30/10—48	34	7	30	164	38	31	— 1
22/ 9—48	35	5 7/12	41	112	3	28	32
21/ 9—48	36	9	38	112	1	24	37
30/ 9—48	37	4 1/12	39[2]	143	30	30[2]	23
29/ 9—48	38	5 1/12	48	159	18	51	— 6
16/10—48	39	12	37	146	22	26	30
28/10—48	40	3 9/12	37	132	18	28	24
20/10—48	41	13	56	117	9	29	48
19/10—48	42	11	28	139	22	27	4
12/ 2—49	43	2 5/12	35	124	13	30	14
13/ 4—49	23	5/12	37	119	5	27	27
9/ 6—49	22	20	57	157		44	23
10/ 6—49	21	20	49	170		27	45
14/ 6—49	44	31	70	154		34	51

[1] Falling time to 140 mg %
[2] Falling time to 120 mg %
[3] Falling time to 110 mg %
[4] The surface area in cm² over 0.1 % where 10 min. = 1 cm (abscissa) and 10 mg % = 1 cm (ordinate).

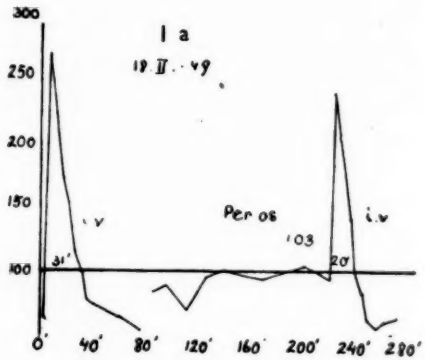


Fig. 32.

tioning. It even appears that especially those peroral experiments which had induced a small rise of the blood sugar level, exercised the strongest influence.

Figure 33 illustrates that at least the height to which the peroral curve rises is not directly proportional to its effect, as one would be inclined to imagine, but rather to the contrary — an inverse correlation seems to be prevailing between the maximum height of the peroral curve and the catalysation induced by it. The correlation coefficient is —0.718 and the corresponding t-value = 5.45. This result is also obtained between the size of the peroral curve

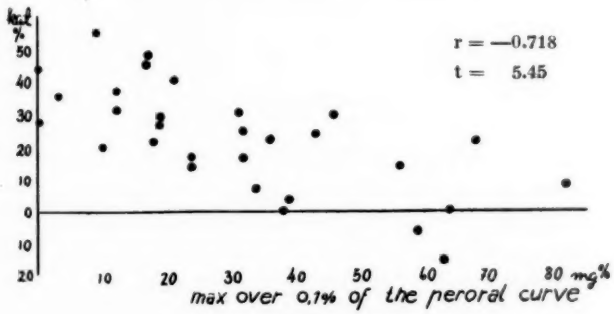


Fig. 33.

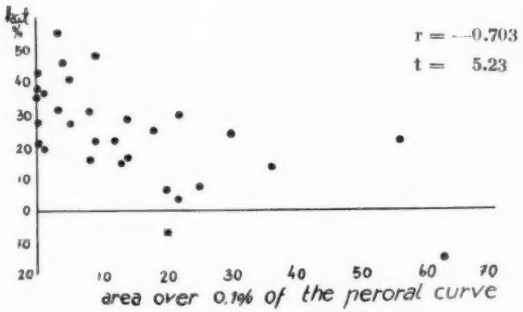


Fig. 34.

surface and the catalysation estimated in the above-described way, (fig. 34) the correlation coefficient being —0.703 and the corresponding t-value = 5.23. The same result is obtained when the catalysation is calculated simply as a shortening in minutes of the second intravenous curve (Fig. 35). The correlation coefficient being now —0.597 and t = 3.94. The mathematical interpretation of these values means that the possibility of obtaining such an *accidental* correlation is less than one in a thousend. Thus it can be assumed as certain that there is an inverse correlation between the height of the perora curve and the catalysation caused by it.

The following figure (36) represent the experiments performed with the same child. It can be noted that this negative correlation also prevails in different tests performed on the same child — the

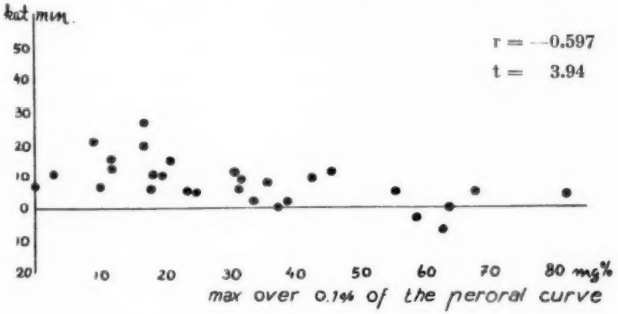


Fig. 35.

less the peroral curve has mounted, the more it catalyses the following intravenous curve.

These experiments having been performed with children, it might be claimed, with regard to the origin of the negative correlation, that the more restless the child, the higher the rise of the peroral tolerance curve, and the slighter the catalysation, also owing to the restlessness of the subject. Yet restlessness does not seem to affect the course of these experiments in any way, nor of the

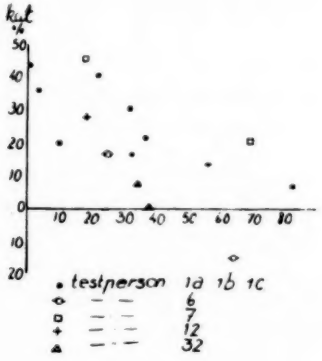


Fig. 36.

other tests described in this work either, since, in the first instance, the children had been in the hospital for a considerable time — minimum one week, and they had already grown accustomed to injections and withdrawals of samples; secondly, the behaviour of the child was carefully recorded and the experiment discontinued as soon as there was even a slightly more pronounced distress.

It would be natural that the same factors which determine the form of the intravenous tolerance curve should be mirrored, at least partly, in the following peroral test, in such a way that if the intravenous curve is short, the peroral curve will be flat, and vice versa. Yet there does not seem to be any correlation between the length of the intravenous and the following peroral curve in these experiments (fig. 37).

The figures prove that the time since the disappearance of hyperglycaemia at the beginning of the second intravenous tolerance test has shown great variability — in some cases slight hyperglycaemia was still prevailing, in several it had disappeared up to 2 hours before the initiation of the intravenous tolerance test. It could be expected that some correlation exists between the duration of normohypoglycaemia preceding the intravenous test

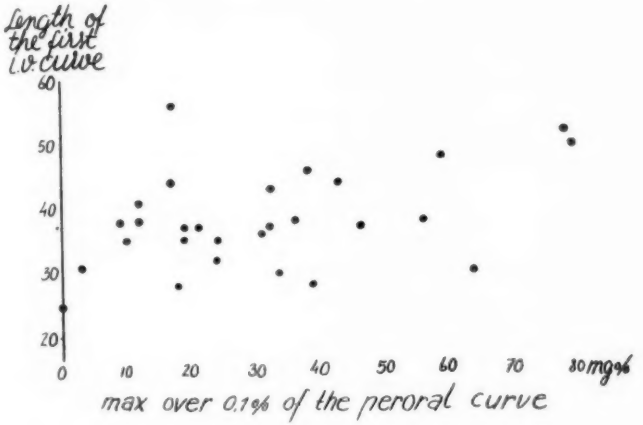


Fig. 37.

and the catalysation, but this does not seem to be the state of things, instead, catalysation seems to persist unchanged for 2 hours at least after the blood sugar has gone down to normal (fig. 38).

Three adults were used as controls and subjected to a similar intravenous-peroral-intravenous test as reported above for children The dose for the adults was 0.3 g/kg intravenously and 2 g/kg by the mouth. In all these tests the peroral dose produced an unmistakable shortening of the intravenous curve. In Fig. 39 the first intravenous curve fell in 57 minutes, the second in 44 minutes. The peroral curve rose to 157 mg%, and had not yet fallen quite to normal before the administration of the second intravenous dose. Shortening of the intravenous curve 13 minutes, i.e. 23%.

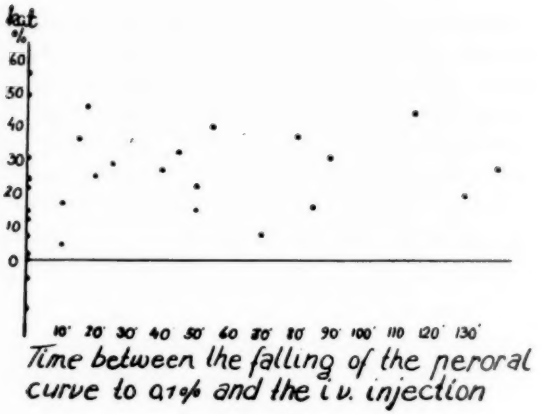


Fig. 38.

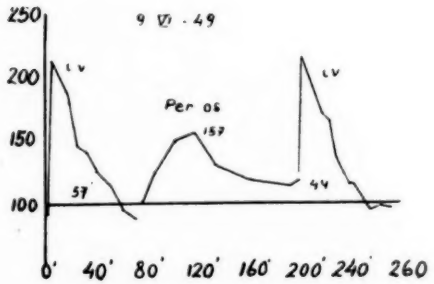


Fig. 39.

In Fig. 40 the first intravenous curve fell in 49 minutes, the second in 27 minutes. The peroral curve rose to 170 mg% and again revealed a rising tendency at the application of the second injection. The shortening of the intravenous curve was 22 minutes, i.e. 45%.

In Fig. 41, the first intravenous curve fell in 70 minutes, the second in 34 minutes. The peroral curve rose to 154 mg% and fell to clearly hypoglycaemic values (73 mg%) when the second intravenous injection was administered. Shortening of the intravenous curve 36 minutes, i.e. 51%.

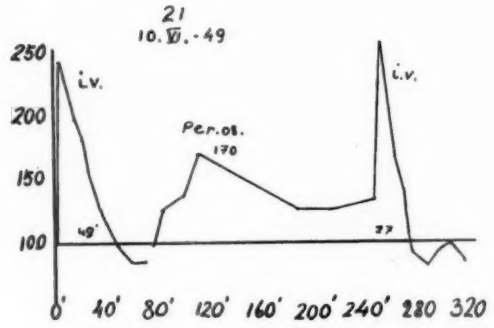


Fig. 40.

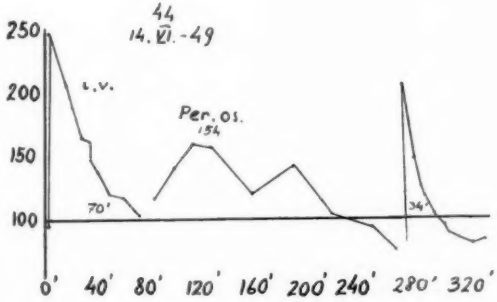


Fig. 41.

These tests performed on adults reveal therefore that there was a fairly pronounced shortening of the second intravenous curve, but it was confined within the same limits as where children were concerned. The peroral curves were all fairly high, in 2 cases much higher than in children with a corresponding degree of catalysation,

Intravenous-intraduodenal-intravenous test.

Therefore, since the peroral glucose tolerance test shortened the following intravenous curve to a very considerable degree in several cases, it was deemed advisable to examine whether this catalysation was still more pronounced if glucose was introduced directly into

the duodenum. The experiments were performed as follows: a thin rubber tube was directed, with the help of X-rays, through the pylorus to a depth of about 10 to 15 cm into the duodenum. When the sound had reached its destination, the intravenous sugar dose was administered. On the completion of the injection an identical amount of glucose solution as already applied per os, was introduced into the duodenum through the rubber tube, after which the intravenous dose was administered again as

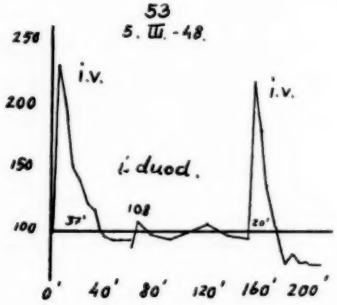


Fig. 42.

described above. Of these experiments 5 were successful with children and 3 with adults. All however complained of nausea after the introduction of the glucose solution into the duodenum, and all brought up part of it as well, in consequence of which the intraduodenal glucose amount was considerably less than 2 g/kg. The position of the sound in the duodenum was checked by means of a radiogram both before and after the experiment. The results were as follows:

Fig. 42: the falling time of the first curve was 37 minutes. Contrary to expection, the intraduodenal glucose tolerance curve hardly rose at all — the main part of the glucose solution having possibly run back into the stomach. The following intravenous curve fell to 0.1% in 20 minutes, the shortening being thus 17 minutes, i.e. 46%.

Fig. 43. The first intravenous curve fell in 41 minutes. The intraduodenal curve rose to 245 mg% and had not yet descended

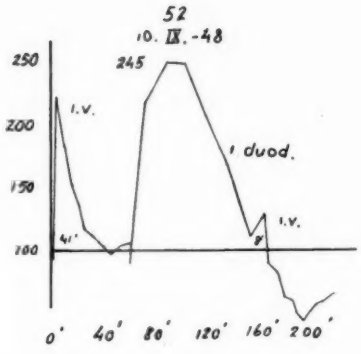


Fig. 43.

fully to normal (109 mg%) at the time the second dose was applied. This fell to 0.1% in 8 minutes and the falling of the curve continued down to 45 mg%. The shortening was 33 minutes, i.e. 80%.

Fig. 44. The duodenal dose was administered before the intravenous curve had gone down to normal. Falling time of the first curve was 41, of the second 29 minutes. The duodenal curve rose to 178 mg%, thereafter exhibiting a strong fall to hypoglycaemic values (49 mg%) and ascending again at the time the second injection was applied. The shortening was 12 minutes, i.e. 29%.

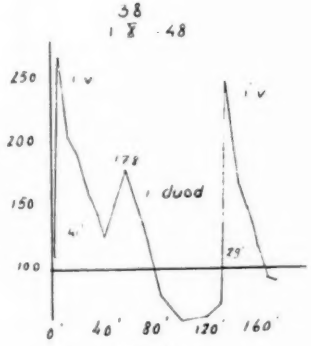


Fig. 44.

Fig. 45. The first intravenous curve fell in 29 minutes. The duodenal curve climbed very high, over 200 mg%. The second intravenous curve had a more rapid fall by 10 minutes. This case exhibited a very pronounced consecutive hypoglycaemia.

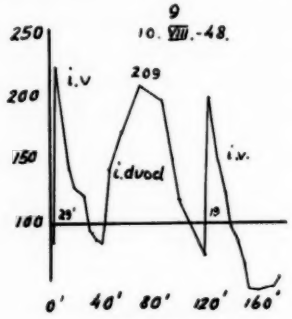


Fig. 45.

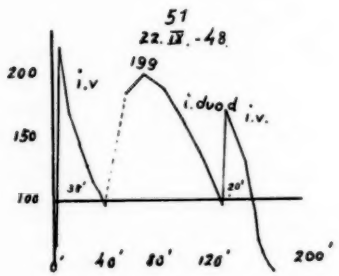


Fig. 46.

Fig. 46. The first intravenous curve fell in 38, the second in 20 minutes. The duodenal curve rose to 199 mg%. The second intravenous curve revealed exceedingly strong consecutive hypoglycaemia (blood sugar 48 mg%). The shortening was 18 minutes, i.e. 47%.

The adult cases yielded the following results:

Fig. 47 and 48. Shortening of the intravenous curve from 75 to 52 minutes, i.e. 30%, and from 62 to 38 minutes, i.e. 39%.

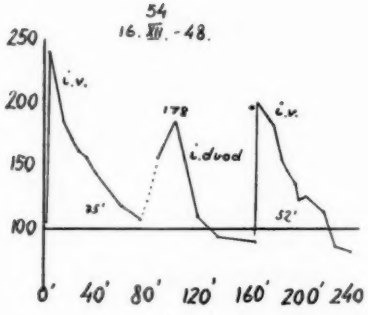


Fig. 47.

Fig. 49. The shortening was from 40 to 28 minutes, i.e. 30%. In these experiments the shortening of the second intravenous curve seems to be still somewhat greater than in the intravenous-peroral-intravenous test. The height of the duodenal curve does not seem to have any correlation to the shortening, probably owing to the experiments being too few.

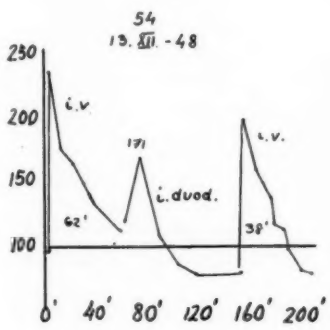
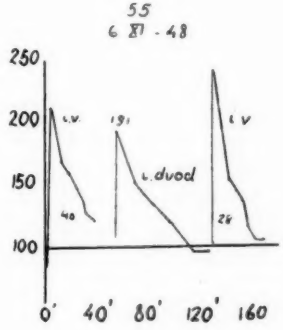


Fig. 48.



Fid. 49.

Results.

In children no Staub effect is found in double intravenous tolerance tests; this is independent of the amount of glucose given and of the interval between the injections, provided that the second tolerance test is performed after the return to normal of the first curve. When the interval between the injections is increased to two and a half to three hours, an inverted Staub effect is produced: the second curve falls more slowly than the first.

In adults, a Staub effect is produced in double intravenous tolerance tests but if the interval between the injections is increased to more than three hours, the Staub effect will also disappear or become inverted.

In double peroral tolerance tests in children the Staub effect is found in most cases, but if the first peroral curve is very low, the Staub effect is often indistinct or inverted — the second curve rises higher than the first.

In intravenous — peroral — intravenous tolerance tests in children a Staub effect is generally found in such a manner that especially after a low peroral curve the intravenous curve is shortened. Also in experiments performed on the same child the maximal height of the peroral curve is regularly in an inverse proportion to the shortening of the subsequent intravenous curve. This shortening of the intravenous curve occurs even if the second intravenous tolerance test is performed more than two hours after the falling of the peroral curve to 0.1 per cent.

In similar experiments on adults the intravenous curve following upon a peroral tolerance test is shortened as much as in children.

In intravenous — intraduodenal — intravenous tolerance tests the duodenal tolerance test causes a still somewhat greater shortening than the peroral tolerance test did in the previous experiments. The height of the duodenal curve does not seem to have any correlation to the shortening of the next intravenous curve, probably owing to the experiments being too few.

Discussion.

In consequence of the foregoing it can be considered certain that in children, contrary to what is the case in adults, an intravenous tolerance test does not accelerate the falling to normal of a subsequent intravenous curve. This phenomenon is independent of the sugar dosage and of the length of the interval between the injections, *provided that the next tolerance test is performed after the return to normal of the first curve.* On page 48 the reason is given why in my opinion a sharp distinction should be made between the Staub effect thus obtained on the one hand, and on the other, a Staub effect found when the next tolerance test is performed *before* the first curve has fallen to normal. One reservation however must be made against the claim that no Staub effect is obtained with double intravenous tolerance tests in children: neither in the intravenous tolerance tests generally used nor in my experiments have any attempts been made to imitate the rate of absorption of glucose from the intestinal tract; it has been injected rapidly »because it is possible to evoke all the characteristic reactions that attend ingestion of glucose by rapid injection of sugar» — page 30. However, to be able to claim with full certainty that an intravenous glucose tolerance test does not produce the Staub effect one ought to perform the intravenous injection slowly; yet this is extremely difficult with children, because they tend to get tired and to grow so restless that the experiment becomes a failure.

A peroral tolerance test, on the other hand, generally lowered the following peroral curve but in many cases both curves are so low that it is difficult to analyse them and draw conclusions about the intensity of the catalysation caused by the first tolerance test. This difficulty largely originates, as expressed on page 32, also

from the fact that the form of the peroral curve is influenced by other factors as well, besides metabolic ones, namely the evacuation rate of the stomach and the absorption rate. Then, should one want to investigate the blood sugar regulation by means of the double tolerance test, the double peroral test is inappropriate, since the »indicator» – the second intravenous tolerance test — also is influenced by other than purely metabolic factors. It is natural that this holds true especially with children who are more sensitive to psychic impulses than adults. The psychic factors affect, as is well known and as is mentioned on page 32, not only the sugar metabolism, but also the stomach evacuation rate and the absorption rate.

Therefore it is possible that the proposed intravenous — peroral — intravenous tolerance test gives a more reliable picture of the acceleration of the blood sugar regulation produced by a peroral tolerance test. The »indicator» — the second intravenous tolerance test — is influenced by metabolic factors only. We do not know with certainty whether this »indicator» is influenced only by the previous peroral test or also to some extent by the first intravenous test. In children, as we know, the intravenous tolerance test did not accelerate the next one of the same kind, instead, it rather retarded it, if the interval was as long as the interval between both intravenous tests in these experiments. In adults the catalyzing influence of the intravenous test also disappeared and was inverted, if the interval was increased to the same length as in these experiments. Therefore it would seem that the shortening of the second intravenous tolerance curve is due to the interposed peroral test.

One would think moreover that if an intravenous tolerance test has no effect on another of the same kind, neither would it affect a subsequent peroral test, A substantiation of this with the aid of the experiments presented above is, however, impossible, since the form of the peroral tolerance curve varies from day to day much more than the form of the intravenous curve.

As already mentioned the second intravenous curve in these experiments usually fell more rapidly than the first and the acceleration was great especially in those cases where the peroral curve was low. In all those experiments where the peroral curve rose to

higher values than 0.155% the acceleration was relatively small, as shown in fig. 33 and 35.

All the theories about the origin of the Staub effect are founded on the facts that when the level of the blood sugar rises, its elimination is accelerated and that this acceleration lasts so long that the second tolerance curve rises less and falls more rapidly than the previous one. According to the oldest theory this rise of the blood sugar level produces the Staub effect by increasing the insulin production. According to HIMSWORTH's earlier theory, it causes an activation of insulin in the liver and by this means a Staub effect. Again, according to the theory of SOSKIN and the later theory of HIMSWORTH, the rise of the blood sugar level brings about the Staub effect by reducing the secretion of blood sugar increasing factor from the hypophysis.

The above results, according to which the intravenous Staub effect is not found in children but is found in double peroral tolerance tests and in intravenous — peroral — intravenous tests, seem to indicate that *in children the Staub effect is not produced by any factor in the region of the general (extra portal) circulation — pancreas, hypophysis, thyroid or adrenals. In that case one would suppose that the high hyperglycaemia following upon an intravenous tolerance test would produce it at least to the same degree as in peroral tolerance tests.* It is probable, however, that if any of the factors in this region cause the Staub effect in adults, it will also cause it in children, although according to my experiments, to so little extent that one sees just a trace of the effect — a minimal positive Staub effect in some of the double intravenous tolerance tests with a short interval, and a slight inverted effect in the long interval tests. This latter phenomenon, the inversion of the intravenous Staub effect when the injection interval is increased can probably be explained by suggesting that when the influence of the catalysator producing the Staub effect has disappeared, the counter regulatory factors dominate; thus after a sufficiently long interval the second curve falls more slowly.

In adults the same phenomenon was found, i.e. that no Staub effect was seen or it was inverted if the injection interval was increased to more than three hours. Therefore it is probable that the difference between the mechanisms producing the Staub effect

in adults and in children is merely that the catalysation caused by the intravenous tolerance test is weaker in children and ends more rapidly than in adults.

Since the Staub effect is produced even without a rise of the blood sugar in the general circulation after peroral administration, the following possibilities as to its mechanism may be conceived:

1. It is induced by the rise of the blood sugar in the liver capillaries. In this case two factors should be borne in mind which probably can contribute towards the manifestation of the Staub effect.

A. First one is bound to think of the well-known regulation mechanism of the liver which, according to SOSKIN, produces the normal blood sugar curve and which, as mentioned on pages 48 and 49, probably can produce a Staub effect also in double intravenous tolerance tests, provided that the second tolerance test is performed before the first curve has fallen to normal or so soon thereafter while the sugar excretion of the liver is not yet on the same level as before the tolerance test. This mechanism may be of importance in the Staub effect, as in a peroral tolerance test absorption takes place long after the blood sugar in the peripheral circulation has fallen to normal, so that the blood sugar of the portal vein at this time still shows supranormal values.

B. The maximal rate of the absorption of glucose is about 2 g per minute. The minute volume of the heart in the resting adult is about four liters, of which a maximum of one quarter, i.e.one liter, passes through the portal vein. According to the recent investigations by estimating the minute volume in man, it is usually under one liter. (BRADLY, INGELFINGER, BRADLY and CURRY in 1945, MYERS in 1947 and BONDY, JAMES and FARRAR in 1949). This would mean that in the liver capillaries the blood sugar rises to 0.2 or even 0.3%. In the dog experiments of CHERRY and CRANDALL the highest glucose concentration obtained from the portal vein was 0.182% but usually much lower, from 0.114 to 0.160%. If we apply these values to man, it would mean that the minute volume of the liver ought to increase during absorption and thus prevent the rise of the glucose concentration of the portal vein to high values — which is fairly certain even without the above cal-

culation, in consequence of our knowledge about the relation of the minute volume to activity of an organ.

If the second tolerance test — be it peroral or intravenous — is performed after the blood sugar has fallen to normal after a *peroral* tolerance test, it is quite possible that the minute volume of the liver is still greater than normal and the sugar concentration of the portal vein increased. Even if the blood sugar in the periphery rises again this time, it is quite possible that the liver still retains more sugar than it receives through the portal vein. Since, after the fall of the peripheral blood sugar the transfer of sugar from the blood into the tissues decreases. If the effect of this phenomenon exceeds that of the return of the liver function to its state before the tolerance test, the peripheral blood sugar rises as in possibility II on page 48, although the sugar curve in the portal vein and in the liver capillaries still resembles possibility IV.

If at this moment an intravenous tolerance test is performed, the curve falls more rapidly than the corresponding curve made before the peroral test, as the liver is functioning more effectively owing to both the above mentioned working mechanism and the increase of the minute volume. The inverse correlation between the maximal height of the peroral curve and the degree of catalysation which it causes can be explained as follows: the more effectively the liver functions after a peroral tolerance test, the less the peroral curve rises and the more it accelerates the subsequent intravenous curve. Thus the peroral Staub effect might be explained mainly with the aid of hemodynamics, without any particular necessity to presume that it is caused by a hormonal factor. The principle is the same as in the bilirubin theory of RÄIHÄ, here only applied to sugar metabolism.

As mentioned above, a Staub effect obtained with intravenous tolerance tests also can be partially explained with the aid of the liver mechanism described on page 48, if the second tolerance test has been performed before the first curve has fallen to normal, or quite soon thereafter. Besides it seems quite possible that an intravenous tolerance test as well would increase the minute volume of the liver, because of the liver being of fundamental importance for the whole regulation of blood sugar. In the experiments of SOSKIN, ESSEX, HERRICK and MANN in 1938 the blood flow of

the liver was still 75—105 min. after an intravenous injection of glucose remarkably increased. However, MYERS, quoted by BONDY, JAMES and FARRAR in 1949, has shown in man, that the injection of glucose intravenously produces a decrease of hepatic blood flow of 10% during the first hour of injection.

2. Another way to explain a Staub effect of this kind, where the peripheral blood sugar does not rise during the peroral test, would be to return to theories according to which the duodenal wall contains some blood sugar lowering substance, whether it has an independent effect or acts by activating insulin. This hypothesis might, equally well as the previous one, explain the fact that the less the peroral curve rose, the more was the subsequent intravenous curve shortened.

It is impossible to decide at this moment to what extent the mechanism founded in hemodynamics described above really causes the Staub effect in children, and whether it is influenced by some hypothetical duodenal factor. In order to investigate this one ought to perform a continuous determination during double tolerance tests both of the minute volume of the liver and the amounts of glucose retained and excreted by it. In the dog experiments of CHERRY and CRANDALL, glucose retention, as the mean of seven experiments, was practically finished 90 minutes after a peroral administration of glucose. The minute volume might, of course, nevertheless be still increased.

There seems to be a contradiction, which is perhaps only apparent, between the degree of the Staub effect obtained with double peroral tolerance tests and that obtained with intravenous — peroral — intravenous tests. The Staub effect produced with double peroral tests was strongest in those experiments where the first tolerance curve was high, and was very weak in those where the maximum of the first curve was below 0.130% (Table II). In the intravenous — peroral — intravenous experiments, again a strong Staub effect was generally obtained, particularly in those experiments where the peroral curve was low. I feel that this contradiction can be explained, at least partly, in the following manner:

If absorption is retarded after the night fast, a second dose might be better absorbed than the first and thus cause a higher rise of the blood sugar than in the first instance, in spite of the first

dose having catalysed the blood sugar regulation. This does not happen if the absorption is maximal already during the first administration and the first curve therefore rises to high level.

If we consider the second possibility, a hypothetic, duodenal hormone, perhaps activating insulin, a certain influence may be exercised by the fact that the insulin effect is greater when the blood sugar is high than when it is low. Much less insulin is needed to lower the blood sugar from 0.160 to 0.150% than from 0.130 to 0.120%. Thus the »indicator» showing the amount of active insulin (maximum of the first curve minus maximum of the second curve) is the less sensitive, the lower the curves are. Therefore it is probable that in the last experiments on Table II more active insulin has been produced than is indicated by the degree of the Staub effect. In other words, even if the first tolerance test had produced a great amount of active insulin, it is possible that its effect is not clearly visible because the »indicator» is not sensitive enough. As a contrast in the intravenous — peroral — intravenous tests, the »indicator», i.e. the second intravenous curve is always equally sensitive.

Perhaps even a third factor can be conceived, which might lessen the Staub effect obtained in double peroral tests. If the first curve is low because a great amount of the blood sugar lowering factor has been produced, it may be possible that this factor has a direct influence upon other incretory glands (the hypophysis or the adrenals) in such a way as to stimulate the excretion of antihormones. In that case this antihormone might dominate during the second peroral tolerance test. In the intravenous—peroral—intravenous test, the high hyperglycaemia produced by the intravenous tolerance test probably prevents the secretion of this hypothetic antihormone.

In this way it might be understood why the Staub effect in the double peroral tests was weak if the curves were low, in contrast to the cases where the intravenous curve served as an indicator.

Thus, it is probable that the Staub effect, in part at least, is due to some factor in the region of the portal circulation — a rise of the minute volume or some blood sugar lowering factor. Furthermore, it is, of course, quite possible that the increase of insulin

secretion and the decrease of the pituitary secretion and perhaps also changes in the adrenal and thyroid secretions, affect it in some way, although they are hardly significant in children, as shown by the absence of the Staub effect in intravenous tolerance tests performed on them.

Above those possible mechanisms have been discussed which may give rise to a Staub effect, especially in cases where it occurs unattended with any remarkable rise of the blood sugar during the first tolerance test. To which process the acceleration of the elimination is due, is hard to say. According to the theory presented above, which is mainly based upon hemodynamics, the glycogen formation rate in the liver is probably stronger during the second than during the first tolerance test.

If we postulate that some kind of duodenal hormone takes part in the production of the Staub effect, it is still harder to answer the above question, since we do not know its mode of action. If it acts in the same manner as insulin or activates the latter, the nearest theory to advance would be an increased rate of glycogen formation and of oxidation in the periphery.

Summary.

27 intravenous double and triple glucose tolerance tests were performed on children 1 to 12 years old. The interval between the injections varied from 40 to 200 minutes and the dose from 0.25 to 2 g per kilogram of body weight. The curves were compared by using the criterion of their »length», i.e. the falling time to 0.1 %. Not a single instance revealed an unmistakably more rapid fall of the second curve to 0.1 % — no Staub effect appeared — provided that the second tolerance test was performed after the return to normal of the first curve. In those experiments where the interval between injections exceeded 60 minutes, a slightly negative Staub effect was obtained as a rule — the second curve descended more slowly than the first.

8 similar experiments were performed on adults. They showed the Staub effect as it is reported in the literature, with the exception of one case, where the first curve fell more rapidly than normal (test person 31). Both those experiments where the interval between injections was very long, revealed as well a negative Staub effect, as in similar tests performed on children.

In 22 double tolerance tests given by mouth the Staub effect appeared as a rule, but there were 5 clearly inverted reactions. This result was obtained particularly in those tests where the first peroral curve was very low.

However the peroral double tolerance test gives an unreliable picture of the blood sugar regulation, since both these tests are affected by several accidental factors, such as the evacuation rate of the stomach and the rate of absorption. If the effect of the peroral tolerance test on the blood sugar regulation is to be studied, the second peroral test is therefore an unreliable »indicator». If, instead of this second peroral test, an intravenous dose is administered and this intravenous curve compared to a similar earlier

one, the change in the blood sugar regulation induced by the peroral curve will in all probability be obtained with greater exactitude. Since the second intravenous test was not greatly affected by the first, this »standard» curve was obtained in several cases by administering the intravenous dose immediately before the peroral one. Of these intravenous-peroral-intravenous tests 30 in all were performed on children aged 1 to 13 years. It was noted as a result that the second intravenous tolerance curve was as a rule shorter than the first, and that this shortening was pronounced particularly in those tests where the peroral dose produced a very slight elevation of the blood sugar. By determining the »catalysation» — shortening of the intravenous curve — both as a percentage and in minutes, it was observed that an inverse correlation exists between the peroral maximum height and the catalysation, and similarly between the area of the peroral curve and catalysation.

It is concluded that the intravenous glucose tolerance test did not accelerate the blood sugar regulation in children, instead, this effect was brought about by the peroral dose, although it produced a much slighter elevation of the blood sugar or none at all. This leads to the conclusion that the mechanism of the Staub effect is influenced by some other agent, besides those already assumed: increased insulin secretion, activation of insulin in the liver, or a reduced glycotropic secretion of the anterior pituitary. These could all be regarded as highly pronounced under the effect of strong hyperglycaemia during intravenous tolerance tests, and can hardly be assumed to occur at all under the influence of those peroral tolerance tests which do not elevate the blood sugar in the extra-portal circulation.

This factor probably exercises its effect during the absorption of glucose or its passage through the portal circulation; therefore it could be either the increase of the minute volume of the liver, or some substance reducing blood sugar, liberated in the intestine during the process of absorption. The Staub effect manifested in children is in all probability mainly produced by this factor. The inverse correlation between the maximum height of the peroral curve and catalysation is easy to interpret in this way, that the stronger this factor, the slighter the rise of the peroral curve, and the greater its shortening effect exercised on the following intravenous curve.

TABLES

TABLE 1

Intravenous two-dose tolerance test in h

Test person No	Birth Day	Date	g/kg	Interval I—II II—III	Fast. Val. mg %	3′	15′	20′	25′	30′	35′	40′
1 a	23/5—47	14/9—48	0.5	I	92	247	182	141	131	110	99	84
				40′	88	228	170	136	129	101	97	83
				II	—	247	184	154	127	120	101	92
					—	247	173	152	122	113	101	88
1 a	23/5—47	7/12—48	2	I	83	>385	>385	>385	>385	347	293	240
				110′	82	>385	>385	>385	>385	344	283	231
				II	—	>385		372	326	295	256	214
					—	>385	378	307	322	293	235	220
1 a	23/5—47	25/2—49	0.5	I	79	236	170	159	124	103	76	74
				190′	—	236	168	156	122	92	76	74
				II	—	265		166	136	113	104	95
					—	261		163		112	95	95
1 c	23/5—47	23/12—48	0.5	I	82	294	200	158	131	112	93	82
				150′	84	278	200	158	124	111	93	82
				II	—	249	169	151	138	114		
				55′	—	243	167	144	138			
				III	—	247	180	173	137	130	103	
					—	249	178	151		130	100	
1 c	23/5—47/	18/1—49	1	I	92	506	345			188	139	122
				70′	92	480	326			181	132	113
				II	—	472	267			143	112	101
				80′	—	468	244			143	112	92
				III	—	500	242			136	139	122
					—	488	225			136	143	120
1 b	23/5—47	16/9—48	0.5	I	97	269	176	149	131	114	106	99
				40′	—		159	147	127	114	106	94
				II	—	269		129	120	101	97	85
				40′	—		167	127	115	99	94	87
				III	—	233	170	149	134	120	112	103
					—	231	161	149	134	120	103	99
1 b	23/5—47	11/12—48	1.6	I	87			355	289	233	201	169
				110′	83	542	410	330	285	216	193	163
				II	—		370	355	299	250	210	192
				110′	—	400	367	330	293	248	218	185
				III	—	602		370	330	299	271	246
					—	578	454	376	314	287	257	231
1 b	23/5—47	9/3—49	0.5	I	78	224	188	155	135	119	106	103
				195′	76	218	171	153	133	114	100	100
				II	—	245	213	200	164	151	126	112
					—	235	239	188	164	146	123	112

1 =

1

test in healthy children and adults

40′	50′	60′	70′	80′	90′	105′	120′	135′	150′	190′	Length I i.v.	Length II i.v.	Length III i.v.	I—II	I—III
											min.				
84											34	35	—	−1	—
83															
92															
88															
240	185	133	56		55						65	72		−7	
231	180	112	51		49										
214	183	144	91		65	76									
220	176	126	87		62	73									
74	76	68			77		76		70	77	29	34		−5	
74	74	70			77		76		68	76					
95	70	70	70												
95	68	63													
82	82	82	75		73			76			33	37	37	−4	−4
82	82	80	75		73			75							
	84														
	84														
	80	76													
	80	76													
122	88	59	63								44	39	46	5	−2
113		58	59												
101	56	45	52	56											
92	56	45	52	54											
122	101	63	56												
120	65	61	56												
99											38	30	40	8	−2
94															
85															
87															
103															
99															
169	103	76	58		71	81					51	69	82	−18	−31
163	107	72	54		71	80									
192	145	117			73	72									
185	142	108			69	69									
246	185	157	114	87	71		83								
231	179	149	108	87	67		83								
103	89	84			76		69		71	69[1]	41	46		−5	
100		78			76		69		71						
112	100	69	73												
112	89	67	73												

[1] = 195′

Table 1

Test person No.	Birth day	Date	g/kg	Interval I—II II—III	Fast. Val. mg %	3′	15′	20′	25′	30′
1 c	23/5—47	1/3—49	0.5	I	83	228	154	134	108	103
				200′	85	229	154	131	105	103
				II	—	247	174	172	120	124
					—	265	172	179	119	112
2	18/6—44	4/3—49	0.5	I	92	250	179	158	135	128
				60′	91	240	163	156	135	126
				II	—	252	179	160	131	131
					—	246	170	156		128

(Cont.)

35′	40
83	6
83	6
94	9
94	7
114	9
114	9
114	8
114	7

(Cont.)

35′	40′	50′	60′	70′	80′	90′	120′	150′	180′	200′	Length I i.v.	Length II i.v.	Length III i.v.	I–II	II–III
											min.				
83	69	67	63			83	78	81		80	30	34		−4	
83	69	62				72	76	76		78					
94	92	71	69	81											
94	78	76	67	76											
114	96	80	85												
114	94	76	80								38	37		1	
114	81	69	65	67											
114	73	67	65	67											

Table 1 (cont.)

Test person No	Birth Day	Date	g/kg	Interval I—II II—IIII	Fast. Val. mg %	3′	15′	20′	25′	30′	35′	40′	5
2	18/6 —44	11/3 —49	0.5	I	78	233	121	107	86	78	76	83	7
				180′	73	233	121	107	78	76	73		7
				II	—	289	126	114		73	76	58	6
					—	278	122	111		73	76	62	6
2	18/6 —44	24/2 —49	0.5	I	95	280	189	152	137	120	104	91	8
				210′	95	268	177	150	134	123	104	91	7
				II	—	292		175	155	137	118	113	9
					—	255		166		137	125	106	9
2	12/6 —44	26/2 —49	0.5	I	87	256	183	172	149	136	119	99	9
				105′	83	250	178	161	149	136	116	98	8
				II	—	221	167	165	154	119	107	99	7
					—	210	167	163	152	117	103	92	7
3	29/12—37	7/3 —49	0.25	I	75	193	143	134	120	120	119	104	9
				100′	81	143	143	132	125	124	111	104	9
				II	—	190	146	148	122	119	110	102	9
					—	188	146	146		110	110	101	8
4	17/9 —43	18/9 —48	0.5	I	92	211	141	115	99	95	75	88	
				75′	—	204	136	108	95	88	75	88	
				II	—	243	148	131	110	83	79	88	
					—	241	146	122	102	79	75	88	
5	31/7 —43	21/12—48	0.5	I	73	258	196	167	149	136	129	94	101
				75′	73	258	181	153	147	136	128		9
				II	—	252	183	158	158	129	114	109	9
					—	252	183	160	147	126	110	107	8
6	31/10—42	23/2 —49	0.5	I	83	256	176	147	121	109	94	91	6
				150′	82	239	172	142	114		94	87	5
				II	—	212	163	158	140	123	101	85	8
					—	203	163	151	138	116	101	87	7
7	2/5 —42	22/2 —49	0.5	I	90	276	188	167	152	127	120	99	7
				180′	90	268	185	165	149	127	117	99	7
				II	—	268	197	172	168	149	134	120	7
					—	270	186	170	141	152	138	120	7
8	23/4 —41	3/6 —49	0.25	I	85	165	151	144	140	115	117	92	8
				65′	82	165	154	144	136	121	101	96	9
				II	—	211	151	140	122	121	107	105	9
					—	194	153	128	117	110	100	89	8
9	6/9 —47	11/8 —48	0.5	I	85	227	152	129	127	94	89	83	
				40′	85	219	147	129	114	92	85	81	
				II	—	231	167	133	117	101	81	78	
					—	219	160	129	110	96	78	78	

[1] = 1

(cont.)

40′	50′	60′	70′	80′	90′	120′	150′	180′	210′	270′	Length I i.v.	Length II i.v.	Length III i.v.	I–II	I–III
											min.				
83	76	86			92	73		86			22	24		−2	
	76	86			83	86		86							
58	67	73													
62	62	73													
91	86	82			82	70	81	81	84		36	44		−8	
91	79	84			81		79	81	88						
113	91	84	82												
106	90	90	77												
99	90	90			89	81[1]					40	38		2	
98	87	83			89	80[1]									
99	74	74	80												
92	74	74	80												
104	97	95			68[2]						46	41		5	
104	97	93													
102	92	84	74												
101	84		74												
88		93	86								24	26			
88		90	84											−2	
88															
88															
94	101	78	71								39	42		−3	
	92	78	64												
109	92	119	76												
107	85	112	74												
91	67	51			69	69	73[3]				33	36		−3	
87	56	53			64	69	73[3]								
85	80	67	67												
87	74	64	65												
99	76	69			83	88	90	90			40	44		−4	
99	74	67			79	88	85	87							
120	79	70	76												
120	74	69	79												
92	82										38	37		1	
96	91														
105	92	82	74												
89	89	82	82												
83											29	29		0	
81															
78															
78															

[1] = 105′ [2] = 100′ [3] = 145′

Table 1 (cont.)

Test person No	Birth Day	Date	g/kg	Interval I—II II—III	Fast. Val. mg %	3′	15′	20′	25′	30′	35′	40′
10	2/8 —47	11/8 —48	0.5	I	85	246		205	147	140	117	
				40′	85	235	219	161	145	131	112	
				II	—	281	197	170	151	131	119	96
					—	259	196	165	147	126	114	
11	26/2 —47	7/1 —49	0.5	I	81	225	151	140	122	101	81	81
				60′	78	219	147	140	122	96	81	78
				II	—	231	157	135	108	87	81	87
					—	225	156	133	105	85	83	87
12	29/7 —43	11/2 —48	0.5	I	85	263	174	150	127	112	90	83
				60′	78	261	168	150	126	106	85	74
				II	—	242	152	131	108	105	88	72
					—	221	152	129	110	101	83	76
13	3/4 —47	9/12—48/	2	I	94	668	462	412	336	277	219	199
				110′	86	652	438	402	336		199	197
				II	—	668	488	385	372	345	301	267
					—	652	484	385	364	344	297	250
14	18/10—46	12/1 —49	1	I	85	>385	309	283	257	235	235	221
				75′	85	373	297	281	257	235	229	218
				II	—	373	305	277	265	235	221	182
					—	364	299	259	257	227	212	181
15	27/6 —46	15/1 —49	1	I	83	385	296			152		101
				105′	83	385	288			149		99
				II	—	361	278			192		142
				120′	—		258			185		141
				III	—	364	274			179		147
					—	358	258			178		147
16	21/1 —17	2/9 —48	0.25	I	101	245	209	149	121	103	98	96
				45′	101	241	171	135	121	103	96	87
				II	—	203	144	128	112	100	87	83
					—	199	144	122	107	94	87	80
17	11/9 —28	2/10—48	0.25	I	96	174	154	138	135	119	118	116
				70′	92	169	149	130	130	114		112
				II	—	176	140	137	126	114	114	109
				60′	—	169	138	135	123	110	109	105
				III	—	167	142	135	129	123	110	105
					—	163	135	133	124	123	105	112

[1] =

(cont.)

40′	50′	60′	70′	80′	90′	105′	120′	150′	210′	270′	Length I i.v.	Length II i.v.	Length III i.v.	I—II	I—III
											min.				
											39	42		−3	
96															
81	79	79													
78	72	76									29	27		2	
87	94	92													
87	90	89													
83	60	60													
74	60	60									32	31		1	
72	56														
76	56														
199	136	106	77		59	70									
197	132	101	70		61						61	77		−16	
267		183	108		65		61	76							
250		177	103[1]		63		61	72							
221	199	163	138												
218	196	156									72[2]	56[2]		16	
182	161	133	96[1]												
181	158	128	85[1]												
101	69	67	60	71	90	94									
99	65	67	60	71	87						40	51	60	−11	−20
142	105	80	69	71	78	94									
141	101	76	67	76	78	89									
147	129	105	80	67	74										
147	119	98	80	67											
96	80														
87	74										33	29		4	
83															
80															
116		96													
112		91									44	37	34	7	10
109		91													
105		85													
105		94													
112		92													

[1] = 75′ [2] = to 0.140 %

Table 1 (cont.)

Test person No.	Birth Day	Date	g/kg	Interval I–II / II–III	Fast. Val. mg %	3′	15′	20′	25′	30′	35′	40′	5
18	17/1 –28	8/6–49	0.3	I	100	250	209	187	166	142	141	130	1
				78′	95	238	206	187	166	141	137	126	1
				II	—	182	150	121	121	123	97	97	
					—	200	137	127	117	117	93	93	
19	5/11–29	17/6–49	0.3	I	102	233	192	177	166	146	127	112	1
				195′	104	235	184	170	152	137	121	111	1
				II	—	214	199	170	154	150	137	139	1
					—	214	197	157	152	148	132	145	1
20	15/8 –25	13/6–49	0.3	I	107	247	182	158	148	130	121	109	
				95′	107	230	164		146	128	119	105	
				II	—	230	182	158	137	124	104	96	
							180	158	135	131	104	96	
21	24/9 –28	8/6–49	0.3	I	110	219	177	175	168	157	144	135	1
				80′	103	214	168	173	159	155	144	133	1
				II	—	233	169	157	142	130	116	103	1
					—	227	162	157	142	130	119	103	
22	15/12–28	15/6–49	0.3	I	106	227	192	185	190	156	132	141	1
				70′	105		192	179	168	152	134	132	1
				II	—	241	194	165	147	138	120	113	
					—	239	190	161	143	134	120	113	

[1] =

(cont.)

40′	50′	60′	70′	80′	90′	120′	150′	180′	210′	270′	Length I i.v.	Length II i.v.	Length III i.v.	I—II	I—II
											min.				
130	112	79	71								54	34		20	
126	112	79	71												
97	91	79	80												
93	84	91	80												
112	104	97	86		88	86	88	88[1]			54	70		−16	
111	102	95	83		90	86	88	93[1]							
139	118	116	102												
145	123	112	102												
109	96	91		86	93[2]						46	37		9	
105	96	89		86	87[2]										
96	86	82	84		93	91	98[3]								
96	86	80	84		91	89	96[3]								
135	130	114	109	100							77	45		22	
133	123	112	107	98											
103	102	91	77	70											
103	93	86	77												
141	111	99	96												
132	110	96	96								58	48			
113	99	78	76		78	87	85		97	92					
113	96	78	76		78	87	85							10	

[1] = 195 [2] = 95 [3] = 165

TABLE 2

Peroral two-dose tolerance test in healthy children

Test person No.	Birth Day	Date	g/kg	Interval I—II II—III	Fast, Val. mg %	5′	15′	30′	45′	60′	75′	90′	120′
1 a	23/5 —47	27/11—48	2	I	93	88	99	146	136	127		113	120
				120′	93	88	111	150	136	124		111	117
				II	—	117	113	131	139	125		77	117
					—	117	106	132	138	125		77	117
1 a	23/5 —47	14/12—48	1	I	92	87	128	135	122	74	87	69	
				90′	90	87	128	133	122	69	87	71	
				II	—	71	71	116	133	99	80	65	
					—	69	76	114	133	99	80	67	
1 a	23/5 —47	15/12—48	2	I	91	117	128	147	169	160		91	121
				120′	84	124	130	147	169	155		87	119
				II	—	111	112	117	128	117		119	96
					—	111	111	110	128	116		119	91
1 a	23/5 —47	6/4 —49	2	I	80	76	94	124	140	124		82	98
				120′	76	76	94	124	138	122		82	96
				II	—	122	89	82	102	105		89	105
					—		85	80	102	105		85	110
1 c	23/5 —47	29/11—48		I	83	90	136	136	141	126		140	83
				120′	83		136	134	138			126	76
				II	—	105	119	133	129	134		88	87
					—	101	106	115	133	133		92	
1 c	23/5 —47	7/4 —49	2	I	74	81	83	99	98	98		94	90
				120′	74		83	103	99	98		92	89
				II	—	112	98	107	98	98		99	92
					—	112	92	101	98	105		98	87
1 b	23/5 —47	/22/11—48		I	83	105	101	103	145	167		140	87
				120′	78	108	103	103	147	163		131	85
				II	—	74	96	124	129	131		112	124
					—	76	98	124	133	124		105	119
1 b	23/5 —47	14/12—48	1	I	87	94	105	133	149	98	108	101	
				90′	83	98	103	133	140	90	103	99	

1 b	23/5 −47	14/12−48	1	I	87	94	105	133	149	98	108	101	
				90′	83	98	103	133	140	90	103	99	
				II	—	92	99	124	137	117	94	80	
					—		99	124	140	117	85		
1 b	23/5 −47	15/12−48	2	I	89	102	105	147	155	169		111	111
				120′	86	102	111	147	158	160		100	109
				II	—	119	124	130	135	123		107	96
					—	117	123	131	133	117		105	93
1 b	23/5 −47	11/4 −49	2	I	80	83	92	105	110	107		105	81
				120′	76	81	89	103	108	103		105	81
				II	—	99	72	69	107	105		117	99
					—	98	72	65	103	101		115	98
6	31/10−42	11/2 −49	2	I	81	88	88	110	119	133		122	103
				120′	—	85	88	108	119	126		120	97
				II	—	134	138	134	115	127		119	114
					—	133	129		120	122		115	
11	26/2 −47	17/1 −49	2	I	85	94	106	104	104	108		103	104
				120′	85	92	103	104	104	112		101	104
				II	—	110	120	125	101	104		94	103
					—	108	124	125	99	99		94	
14	18/10−46	17/1 −49	2	I	90	106	99	103	115	118		124	125
				120′	85	106	99	101	113	113		129	117
				II	—	124	110	86	122	120		104	103
					—	122	108	86	122	120		108	99
23	22/10−48	12/4 −49	2	I	96	115	126	105	115	110		110	98
				120′	94	115	122	103	114	109			96
				II	—	115	121	98	82	89		91	91
					—	114	121	94	82	87		90	91
24	27/1 −48	8/4 −49	2	I	72	88	99	97	90	90		115	83
				120′	68	83	97	97	88	90		112	83
				II	—	90	99	124	110	94		134	101
					—	88	101	120	110	94		136	99
25	12/9 −47	11/1 −49		I	76	83	100	137	140	116		107	116
				120′	76	83	103	142	142	116		107	116
				II	—	126	145	151	121	109		94	123
					—	123	149	147	121	110		92	

TABLE

Test person No.	Birth Day	Date	g/kg	Interval I—II II—III	Fast. Val. mg %	5′
26	26/2 —47	9/4 —49	2	I	94	110
				120′	94	112
				II	—	83
					—	83
27	29/10—47	30/11—48		I	94	116
				120′	92	116
				II	—	99
					—	99
28	17/8 —47	13/1 —49	2	I	63	54
				120′	62	54
				II	—	101
					—	110
29	19/10—45	29/11—48	2	I	96	94
				120′	96	105
				II	—	96
					—	96
30	24/6 —41	27/10—48		I	80	80
				120′	75	80
				II	—	128
					—	128
31	27/9 —41	21/10—48		I	85	94
				120′	84	94
				II	—	118
					—	112

2 (Continued)

15′	30′	45′	60′	75′	90′	120′
138	138	131	128		110	107
137	135	128	123		107	103
92	78	85	96		105	78
92	74	83	89		101	80
131	112	101	99		101	99
131	112	97			99	97
101	108	103	106		103	92
92	103	103	105		103	99
76	98	101	105		89	121
78	98	99	105		81	116
114	112	96	99		90	87
119	112	98	96		90	87
110	129	126	112		90	99
106	129	119	105		85	96
94	99	92	103		81	99
89	94	96	99		81	94
93	131	149	155		124	126
96	131	149			119	123
119	126	112	103		100	91
119	126	111	103		96	94
123	125	107	109		116	96
123	125	107	107		114	94
123	114	116	102		128	116
125	109	116	103		128	116

TABLE 3

Intravenous — per os — intravenous — test in

Test person No	Birth Day	Date	I. i.v. per os II i.v.	Fast. Val. mg %	3′	5′	15′	20′	25′	30′	35′	40′	45′	5
1 a	23/5—47	8/11—48	I i.v.	82		284	243	211	208	179	152	141		
			40′	—		302	232	204	195	175	150	134		
			Per. os.	—		122	102			81			86	
			145′	—		122	101			81			84	
			II i.v.	—		274	188	159	111	92	77	59		
				—		266	177	152	110	90	70	59		
1 c	23/5—47	23/11—48	I i.v.	78		263	197	168	143	126	122	114		
			60′	74		246	186	167	143	120	114	105		
			Per. os.	—		103	124			133			76	
			120′	—		103	122			131			79	
			II i.v.	—	277		201	158	140	119	106	88		
				—	275		194	158	133	177		79		
1 b	23/5—47/	24/11—48	I i.v.	84	300		220	206	170	152	143	130		1
			60′	82	288		209	195	166	150	136	123		
			Per. os.	—		123	170			188			115	
			120′	—		122	175			177			107	
			II i.v.	—	294		215	166	155	144	118	109		1
				—	286		209	161	150	137	111	108		
1 c	23/5—47	1/12—48	I i.v.	72	275		197	170	140	116	107	85		
			60′	69	263		190	163	138	112	98	83		
			Per. os.	—		103	131			135			83	
			130′	—		105	131			128				
			II i.v.	—	307		196	136	103	78	67	53	53	
				—	285		187	143	103	85		49		
1 b	23/5—47	2/2 —49	I i.v.	94	251		170	149	131	122	108	103		
			60′	89	235		170	145	129	121	105	94		
			Per. os.	—		94	121			135			103	
			120′			98	129			138			105	
			II i.v.	—	349		210	178	128	101	96	94	99	
				—	333		192	172	124	101	96	92	107	
1 c	23/5—47	19/2 —49	I i.v.	73	249		190	158	129	117	100	87		
			60′	72	245		183	149	123	115	100			
			Per. os.	—		67	92			94			114	
			175′	—		67	92			94			107	
			II i.v.	—	264		181	131	110	94	87	78		
					241		167	129	110	94	80	78		

3

test in healthy children and adults

45′	50′	60	65′	70′	75′	80′	90′	120′	135′	150′	155′	175′	180′	Length I min.	Length II min.	Kat. %
														39[1]		
86		90					86	77								44
84		93					86	77								
															22[1]	
	79	74												43		
	81	67														
76		114					131	90								16
79		114					124	88								
	74	81													36	
	74															
	100	86												50		
	99	79														
115		68					100	88								8
107		72					91	88								
	100	99													46	
	95	91														
	65	56												36		
	60	53														
83		81					101	96								31
		72					94	96								
53	80														25	
	67															
	81	69												38		
	78	69														
103		103					87	112								21
105		117					85	109								
99															30	
107																
	64	58												35		
	64	56														
114		89					94	92		55		49				20
107		85					83	92		53						
	71														28	

[1] Falling time to 140 mg%.

TABLE

Test person No.	Birth Day	Date	I i.v. Per. os. II i.v.	Fast. Val. mg %	3′	5′	15′	20′	25′	30′	35′	40′
1 a	23/5 −47	18/2−49	I i.v.	72	270		174	154	120	101	81	76
			75′	63	256		170		111	101	81	74
			Per. os.	—		87	94			74		
			145′	—		85	87			69		
			II i.v.	—	245		145	101	92	61	56	58
				—	227		143	99	78	60	51	52
1 b	23/5 −47	21/2−49	I i.v.	84	298		209	162	151	124	109	89
			60′	76	288		184	162	144	121		91
			Per. os.	—		103	121			112		
			150′	—		100	121			102		
			II i.v.	—	243			112	89	69	66	64
				—	226			112	89	69	64	60
2	18/6 −44	7/3−49	I i.v.	85	273		149	123	107	100	89	85
			60′	83	256		145	123	100	97	83	85
			Per. os.	—		100	103			105		
			155′	—		101	103			107		
			II i.v.	—	264		130	116	87	71	58	58
				—	262		130	112	80	67	58	56
6	31/10−42	7/2−49	I i.v.	87	257		212	199	176	176	160	149
			60′	87	248		208	188	176	167	156	143
			Per. os.			126	136			158		
			120′	—		124	140			163		
			II i.v.	—	324		256	257	227	212	194	181
				—	307		248	248	221	212	197	185
6	31/10−42	17/2−49	I i.v.	76	269		184	145	132	113	92	81
			90′	76	269		166	141	131	101	88	74
			Per. os.	—		76	106			117		
			165′	—		83	106			110		
			II i.v.	—	259		148	113	106	83	88	70
				—			143	110	101	83		70
7	2/5 −42	16/2−49	I i.v.	65	246			165	156	150	141	117
			60′	63	235			165	154	149	132	120
			Per. os.	—		94	138			168		
			135′	—		97	138			165		
			II i.v.	—	284			179	154	129	122	111
				—	284			174	147	124	122	108
7	2/5 −42	14/2−49	I i.v.	86	284		213	168	157	138	124	111
			60′	84	282		202	173	155	138	124	104
			Per. os.	—		63	99			119		
			150′	—		66	97			115		
			II i.v.	—	294		170	120	99	88	88	88
				—	286		150	115	95	88	88	90

3 (Continued)

45′	50′	60′	65′	70′	75′	80′	90′	120′	135′	150′	155′	165′	180′	Length I min.	Length II min.	Kat. %
		63			54									31		
		63			54											
101		101					99	106	49[1]							36
94							92	101	29[1]							
	63														20	
	63															
	69	58												37		
	60	58														
98		107					103		78	58						41
89		107							76							
58															22	
55																
	85	82												28		
	83	76														
109		123					109	98			98					21
107		114					101	94								
	56														22	
	57															
	123	119												42		
	119	121														
161		156					154	156								—17
165							158	158								
	138	133													49	
	140	135														
	68	54		56		54	63							32		
	63	64		58		56	59									
115		129					97	92				74				16
112		120					97	94				72				
	63	63													27	
	63															
	117	87												45[3]		
	117	96														
170		158					129	147	113							11
167		158						150	110							
108															40[3]	
105																
	108	65												44		
	106	66														
95								99[4]								45
95								95[2]								
	95														24	
	88															

[1] = 105′ [2] = 140′ [3] Falling time to 110 mg%.

TABLE 3 (Cont

Test person No.	Birth Day	Date	I i.v. Per. os. II i.v.	Fast. Val. mg %	3′	5′	15′	20′	25′	30′	35′	40′	15′	5
12	29/7 −43	8/2−49	I i.v.	84	243		164	153	144	132	109	97		8
			60′	79	232			150	146	130	108	95		8
			Per. os.	—		82	104			102			130	
			135′	—		82	108			97			129	
			II i.v.	—	306		175	136	129	111	93	77		5
				—			170	134	127	108	91	75		5
12	29/7 −43	10/2 −49	I i. v.	85	307		180	162	133	118	103	92		6
			60′	83	295		171	147	130	116	98	91		5
			Per. os.	—		71	71			98			119	
			175′	—		71	78			100			119	
			II i.v.	—	227		140	103	103	82	71	56		5
				—	212		138	101	98	83	69	56		5
15	27/6 −46	28/1−49	I i.v.	87	283		167	135	92	83	71	72		7
			60′	85	277		163		92	81	69	69		7
			Per. os.	—		65	76				85		94	
			120′	—		65	83				81		90	
			II i.v.	—	242		129	85	81	67	58	65		6
				—	205		119	83	71	65	56	62		6
21	24/9 −28	10/6−49	I i.v.	104	242		199	179	157	137	128	116		9
			70′	102	242		199	173	155	137	126	111		9
			Per. os.	—		102	123			139			170	
			175′	—		102	128						170	
			II i.v.	—	254		168	137	109	93	86	84		9
				—	235		150	132	105	91		79		9
22	25/12−28	9/6−49	I i.v.	91	210		185	176	146	137	139	128		11
			70′	91	208		185	180	144	153	169			11
			Per. os.	—		103	124			149			155	
			67′	—		108	131			149			160	
			II i.v.	—	212		173	164	135	121	108	108		9
				—	208		167	158	135	119	107	101		9
22	25/12−28	21/6−49	I i.v.	100	217		176	151	153	145	136	127		11
			70′	—	219		174	151	149	145	122	122		11
			Per. os. water	—		85	82			83			85	
			135′	—		85	80			82			83	
			II i.v.	—	251		167	162	151	147	136	127		11
				—	209		163	160	145	145	127	122		11
23	22/10−48	13/4−49	I i.v.	92	257		172	142	119	112	103	99		8
			60′	90	254		165	138	119	112	107	89		8
			Per. os.	—		80	107			122			108	
			125′	—		81	107			117			105	
			II i.v.	—	273		179	147	104	99	83	62		6
				—	261		167	147	102	92	81	62		8

3 (Continued)

45′	50′	60′	65′	70′	75′	80′	90′	120′	135′	150′	155′	175′	180′	Length I min.	Length II min.	Kat. %
	88	61												38		
	88	59														
130		136					130	155	139							13
129		136					129	157	139							
	55														33	
	57															
	60	51												35		
	58	47														
119		119					107	105		109			64			29
119		112						109		107			65			
	53														25	
	55															
	78	65												25		
	78	63														
94		76					80	83								28
90		76					83	78								
	65[1]														18	
	65[1]															
	99	88		88										49		
	97	88		86												
170		162					146	128		128			135			45
170		162					146	123		125			132			
	95	102	86												27	
	93	102	88													
	115	95		87										57		
	115	95		86												
155		130					121	117	119[2]							23
160		128					115	122	124[2]							
	96	100	89												44	
	93	94														
	115	96		101										57		
	112	94		101												
85		83					89	89	78							—5
83		83					91	89	80							
	119	100	101												60	
	117		101													
	85	94												37		
	85	94														
108		107					103		98							27
105		99					99		98							
	62	69													27	
	85	69														

[1] = 55′ [2] = 127′

TABLE 3 (Cont

Test person No.	Day Birth	Date	I i.v. Per. os. II i.v.	Fast. Val. mg %	3′	5′	15′	20′	25′	30′	35′	40′	45′	50
32	17/6—39	4/2 —49	I i.v.	88	270		184	179	148	132	120	115		93
			60′	89	258		182	171	144	134	120	155		90
			Per. os.	—		99	109			125			141	
			135′	—			109			120			136	
			II i.v.	—	306		182	155	143	118	113	104		99
				—	302		173	164	139	118	117			
32	17/6—39	31/1 —49	I i.v.	97	258		144	137	118	100	88	73		70
			60′	95	247		141	137	118	100	84	71		
			Per. os.	—	93		120			137			115	
			120′	—	91		124			132			113	
			II i.v.	—	262		164	130	118	93	93	93		
				—	245			123	116	93	93	91		
33	2/6—46	3/2 —49	I i.v.	78	254		187	152	117	114	110	96		76
			60′	78	248		183	151	117	105	101	94		74
			Per. os.	—		78	105			110			107	
			120′	—		76	101			108			107	
			II i.v.	—		225	110	80	63	53	54	67	80	
				—		212	105	80	62	54	54		78	
34	18/6—41	30/10—48	I i.v.	93	152		134	122	111	106	92	86		
			60′	88	148		134	120	113	99	86	86		
			Per. os.	—		97	136			164			157	
			130′	—		101	143			164			157	
			II i.v.	—	209			136	119	106	93	81		
			75′	—	195			131	113	99	93	81		
			III i.v.	—	163		124	110	95	90	79	88		
				—	164		117	101	95	90	83	81		
35	16/2—43	22/9 —48	I i.v.	89	308		192	173	149	133	107	105		
			60′	85	302		187	169	147	130	105	100		
			Per. os.	—		85	100			78			109	
			120′	—		85	89			71			105	
			II i.v.	—	298		171	147	112	96	89	84		
				—	286		169	140	112	94	85	87		
36	28/10—38	21/9 —48	I i.v.	92	285		205	179	170	128	107	96		
			60′	90	275		199	174	170	121	107	90		
			Per. os.	—		80	89			112			94	
			120′	—		80	89						94	
			II i.v.	—	248		161	126	94	99	80	76		
				—	244		161	124	90	94	80	72		
37	23/8 —44	30/9 —48	I i.v.	100	317		218	194	169	164	140	120		
			40′	100	307		214	174	169	158	137	111		
			Per. os.	—		109	107			114			126	
			120′	—		103	112			116				
			II i.v.	—	334		178	162	149	121	116	107		
				—	315		178	160	147	120	112	105		
38	8/8 —43	29/9 —48	I i.v.	87	250		195	179	165	145	136	114		
			60′	87	248		192	177	163	141	133	112		
			Per. os.	—		74	126			159			126	
			90′	—		74	122			159			124	
			II i.v.	—	254		179	165	154	145	126	119		
				—	240		172	174	152	143	124	118		

3 (Continued)

45′	50′	60′	65′	70′	75′	80′	90′	120′	135′	150′	155′	165′	175′	Length I min.	Length II min.	Kat. %
	93	73												46		
	90	73														0
141		111					108	109	117						46	
136		109					104	106	123							
	99															
	70	80												30		
		81														7
115		111					120	120								
113		109					120	121							28	
	76	72												38		
	74	72														55
107		99					90	105							17	
107		99					85	105								
80																
78																
		75												31		
		74														
157		163					90	122								0
157		161					90	122								
		83													31	
		86														
		82												41		
		75														
109		112					87	100								32
105		112					87	94								
															28	
		76												38		
		74														
94		98					105	94								37
94		98					101	94								
															24	
														39[1]		
126		144					128	120								23
		142					128	121								
															30[1]	
		81												48		
		79														
126		112					110									—6
124		112					110									
		90													51	

[1] Falling time to 120 mg%.

TABLE 3 (Con

Test person No.	Birth Day	Date	I i.v. Per. os. II i.v.	Fast. Val. mg %	3′	5′	15′	20′	25′	30′	35′	40′	45′	5
39	3/6 −36	16/10−48	I i.v.	87	180		140	131	123	116	107	95		
			60′	87	175		139	124	119	112	102	93		
			Per. os.	—		84	98			121			148	
			140′	—		82	98			119			144	
			II i.v.	—	175		117	117		91	84	84		
				—	178		119	116	104	89	82	84		
40	23/1 −45	28/10−48	I i.v.	84	188		146	125	119	117	102	92		
			90′	—	173		138	125	119	119	106	92		
			Per. os.	—	111		110			132			129	
			135′	—	111		106			132			125	
			II i.v.	—	191		131	117	110	97	93	93		
				—	193		125	113	106	90	93	88		
41	1/7 −35	20/10−48	I i.v.	96	172		156	154	143	133	126	120		
			60′	92	170		156	153	138	135	126	120		
			Per. os.	—		92	103			110			94	
			120′	—		92	101			110			92	
			II i.v.	—	199		134	126	112	99	96	92		
			85′	—	183		133	120	110	97	94			
			III i.v.	—			129	120	99	103	105	103		
				—			129	112	99	105	103			
42	26/7 −37	19/10−48	I i.v.	82	166		130	116	105	96	91	85		
			60′	82	166		126	114	105	94	87	84		
			Per. os.	—		85	114			133			138	
			130′	—		85	116			135			140	
			II i.v.	—	192		140	124	105	91	84	82		
			75′	—	189		144	121	105	89	76	78		
			III i.v.	—	156		116	109	98	87	85	64		
				—	155		109	105	93	82	82	64		
43	12/9 −46	12/2 −49	I i.v.	82	256			147	118	110	105	71		
			60′	76	250			142	118	112	100	69		
			Per. os.	—		80	109			123			116	
			180′	—		73	114			126			114	
			II i v.	—	229		138	118	112	105	89	87		
				—	216		140		109	98	89	85		
44	16/11−17	14/6 −49	I i.v.	98	245		204	187	165	162	146	137		1
			70′	98	236		200	185	165	160	146	135		1
			Per. os.	—		96	115			140			155	
			200′	—		100	119			138			153	
			II i.v.	—	204		146	126	115	105	98	91		
				—	194		144	126	114	100	98	89		
45	16/5 −38	26/6 −49	I i.v.	91		219	169	151	135	117	116	100		
			70′	89		219	167	149	135	117	107	99		
			Per. os. water	—		78	75			80			82	
			130′	—		78	73			84			82	
				—	251		171	155	153	140	124	112		
			II i.v.	—	247		166	153	148	126		110		

3 (Continued)

45′	50′	60′	65′	70′	75′	80′	90′	120′	130′	150′	155′	175′	180′	Length I min.	Length II min.	Kat. %
		91												37		
		89														
148		137						110								30
144		135					112	110							26	
		86					97							37		
		86					85									
129		113					119	88								24
125		111					119	88							28	
		96												56		
		96														
94		117					96	119								48
92		117					108	122								
		92													29	
		99														
		80												28		
		75														
138		124						102								4
140		121						100								
		75													27	
		75														
	69	65												35		
	60	65														
116		116					94	123		62			51			14
114		110					91	124		60			49			
	85														30	
	85															
	117	112		102										70		
	114	108		103												
155		153					122	142		107			94			51
153		151					122	133		105			94			
	84	82	84												34	
	84	82	82													
	80	82		78										40		
	82	78		78												—15
82		82						86	95							
82		80						89	93						46	
	98	89	91													
	93	87														

TABLE 3 (Con

Test person No.	Birth Day	Date	I i.v. Per. os. II i.v.	Fast. Val. mg %	3′	5′	10′	15′	20′	25′	30′	35′	40′	4
46	29/3 –46	16/1–48	I i.v.	96	271		228	198	155	147	131	117	102	
			60′	94	263		217	191	153	138	126	114	94	
			Per. os.			108		129			140			1
						108		129			137			1
46	29/3–46	17/1–48	I i.v.	77	244		186	168	148	139	116	102	89	
			60′	79	231		180	166	148	132	114	95	86	
			Per. os.			91		112			132			1
						89		109			126			1
46	29/3–46	11/2–48	Per. os.	96		119		130			134			
			90′	—		118		128			134			
			II i.v.		210		171	130	99	77	68	66	77	
					193		159	118	95	77	68	66	72	
47	6/6–41	27/7–48	I i.v.	90	212		188	174	161	147	134	129	115	1
			60′	85	212		183	168	158	140	134	127	112	1
		30/7–48	Per.os. 120′	90		99		103			124			1
			II i.v.		163		134	105	90	87	78	76	74	
48	29/3–37	9/3–48	I i.v.	91	245		216	198	180	165	145	138	128	1
			60′	94	241		214	196	172	162	140	135	125	1
			Per. os.			101		144			91			
						103		142			92			
48	29/3–37	8/3–48	Per. os.	92		115		154			129			1
			60′	87		122		152						1
			II i.v.		269		216	168	172	129	112	105	108	
					261		210	167	168	124	108	105	101	
49	13/6–20	28/3–47	I i.v.	102	192		248	225	212	193	162	141	132	1
			60′	88	288		242	223	212	183	159	139	126	1
			Per. os.			99		130			114			
			150′			100		126			112			
49	13/6–20	30/3–47	Per. os.	87		94		138			106			1
			150′	89		98		131			108			1
			II i.v.		317		277	221	187	156	129	108	87	
					317		261	217	185	156	126	105	87	
50	12/1–19	30/1–48	I i.v.	91	237		205	189	183	180	167	135	140	1
			60′	91	234		207	183	182	171	165	133	137	1
			Per. os.			105		115			126			1
						102		115			124			1
50	12/1–19	19/1–48	Per. os.	77		79		111			139			1
			120′	75		86		111			141			1
			II i.v.		199		146	126	95	88	81	81	77	
			60′		189		143	118	97	84	81	81	75	

3 (Continued)

40′	45′	50′	60′	70′	75′	80′	90′	120′	140′	150′				Length I min.	Length II min.	Kat. %
102	91	84	89											40		
94	87	85	84													
	129		110				91	93		85						50
	128		105				96	87		85						
89	77	64	57											34		
86	75	64	55													
	107		102				102	77		62						
	107		102				100	73		59						
	109		104													
			100													
77	77	70	81												20	41
72	70	68	77													
115	106	96	97													
112	101	94	99											47		66
	130		127				117	97								
74	76	69	65												16	
128	112	107	91											54		
125	112	105														
	65		100				110	112		82						
	69		107				110	112		78						22
	106		103													
	106		99													
108	97	101	88													
101		96	88												42	
132	119	104	91													
126	111	102	97											53		
	97		99				105	111		90						
	95		102				111	102		99						
	115		113				92	89		89						30
	120		112				96	89		82						
87	89	94	69		71										37	
87	85	94	69		67											
140	135	119	110											62		
137	126	122	115													
	129		115				112	108		73						
	119		115				107	112		67						
	148		135				100	79								68
	146		130				100	79								
77	61	63	68												20	
75	61	61	66													

TABLE 4

Intravenous — intra-duodenal — intravenous test in healty children and adults

Test person No.	Birth Day	Date	I. i.v. I. duod II i.v.	Fast. Val.	3′	: 5′	10′	15′	20′	25′	30′	35′	40′	45′	50′	60′	70′	75′	80′	90′	105′	Length I min.	Length II min.	Katal %
9	6/9—47	11/8 —48	I i.v.	85	227			152	129	127	94	89	83									29		
			40′	85	219			247	129	114	92	85	81											
		10/8—48	I. duod.	82		134		171			215			197		120			77					35
			80′	82		144		189			204			189		111			68					
			II i.v.		209		157	127	104	86	66	48	43	45	48	54								
					189		137	122	91	79	61	43	45	46	48	57							19	
38	8/8—43	1/10—48	I i.v.	112	268			203	191	175	157	146	130									41[a]		
			40′	111	266			198	191	167	159	148	123											
			I. duod.	—				178			138			82		49		58		75				29
			90′	—				178			135			76		49		58		69				
			II i.v.	—	254			167	151	133	116	98	95											
				—	237			167	144	133	114	94											29[a]	
51	17/8—46	22/9 —48	I i.v.	71	222			171	174	13	119	109	94									38		
			40′	64	220			164	140	12	112	107	94											
			I. duod.	—				178			198			187		156		137		94				47
			90′	—				180			200			187		165		131						
			II i.v.	—	173			124	102	91	67	53	46											
				—	169			117	102	78	66	51											20	
52	27/7—48	13/9 —48	I i.v.	96	221		190	153	138	114	110	107	103	100	100	101						41		
			60′	94	218		172	149	140	118	109	107	101	94	98	100								
			I. duod.	90		129		204			247			143		208		170		113				80
			90′	90		138		228			243			241		197		166		106				
			II i.v.		129		93	79	65	57	50	45	52	54	59	65								
					129		92		61	56	50	45	50	54	57	63							8	

53	24/5—48	5/8 —48	I i.v.	100	230		187	151	140	123	119	102	98	94	94	94					37		
			60′	96	226		182	149	140	121	116	102	98	93	93	91							
			I. duod.	87		112		98			96			99		108		98		96			46
			90′	85		105		96			92			98				96		90			
			II i.v.		223		178	136	107	87	74	80	74	74	71	71							
					214		176	129	98	81	71			74								20	
54	31/10—27	13/12—48	I i.v.	105	240			179	161	179	154	145	133			114					62[1]		
			60′	98	235			185	169	156	151	145	135			110							
			I. duod.	—	119			176			114			96		83				85			39
			90′	—	124			167			107			87		83				85			
			II i.v.	—	197			160	149	140	128	110	108		87	83							
				—	196			154	145		125	114	108			81						38[1]	
54	31/10—27	16/12—48	I i.v.	107	241			181	174	160	156	154	144		128	119		110			75[1]		
			75′	100	237			180	171	167	156	145	136		131	117							
			I. duod.	—				156			178			112		96				92			30
			90′											107		94				89			
			II i.v.	—	207			180	135	147	136	126	126		112	94		91					
				—	190			176		140	133	117	126			94		92				52[1]	
55	9/11—22	6/11—48	I i.v.	89	212			165	158	147	133	130	121								40[2]		
			50′	89	205				153	147	135	124	121										
			I. duod.	—				193			153			133		116		94		94			30
			90	—				190			151			131		116		94		92			
			II i.v.	—	244			154	142	133	114	109	107										
				—	223			151	138	131	114	103	105									28[2]	

[1] Falling time to 110 mg%
[2] » » » 120 mg%

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OF HELSINGFORS, HEAD: PROFESSOR K. O. RENKONEN

THE TUBERCULIN REACTION IN DIFFERENT PARTS OF THE SKIN AND THE SENSITIVITY IN RHEUMATOID ARTHRITIS

With special reference to the technical error in the Mantoux reaction

BY

OLE WASZ-HÖCKERT

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To my wife

PREFACE

The theme of this work was suggested by Professor K. O. Renkonen in 1946. It is a continuation of a small investigation which I did in 1945 under his supervision. My work has been interrupted several times owing to various circumstances, yet Professor Renkonen's valuable advice and friendly criticism has never failed me.

To Professor Arvo Ylppö, my present Chief, I proffer my sincere thanks for his interest in my studies. I am indebted to him for the financial aid granted to me by the Foundation for Pediatric Research.

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Helsingfors, April, 1950.

Ole Wasz-Höckert

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Introduction

Since KOCH's discovery of tuberculin, in 1890, and especially since PIRQUET, in 1907, demonstrated its significance as a diagnostic aid in tuberculosis, this substance has become one of our most important medical adjuvants. In tuberculosis of children tuberculin is of very great value.

The world literature on tuberculin problems is almost unsurveyable now. Assessment of the reports is rendered difficult owing to a great number of the papers being based on unreliable technique, and the authors seem to have drawn too wide, speculative conclusions. The results of the workers who have studied the same theme are therefore sometimes quite contradictory.

In the present work the author has studied the influence of dermo-anatomical factors on sensitivity to tuberculin, as well as the tuberculin sensitivity of patients with rheumatoid arthritis.

The Mantoux reaction in capillary-microscopical and histological light

The local course of events after intracutaneous tuberculin injection (Mantoux) has been studied »capillaroscopically» (microscopically) in vivo by LADE (1921) and histologically by BANDLER and KREIBICH (1907).

LADE made an intradermal injection strictly lege artis 0.1 mg. old tuberculin. He studied the course of the reaction under a binocular microscope, with a magnification of 35, using a drop of liquid paraffin on the skin and in direct sunlight. He describes his observations in detail:

After 6 hours the actual tuberculin reaction becomes visible. The flush increases, the vessels dilate and become less distinct. In the border zone the capillary loops are more defined.

The process develops and extends. The redness spreads to the periphery and the vessels become indistinct, and after 12 hours well filled vessels are seen in the border zone.

After 36 to 40 hours the reaction is at its maximum. The skin is attenuated.

After 2 ½ to 3 days the periphery becomes lighter.

On the 3rd to 4th day scaling sets in.

In BANDLER and KREIBICH's histological investigation the following was observed:

Primarily a minimal capillary injury resulting in effusion of blood in the injection channel. The adjacent vessels become dilatated. (An exactly similar reaction was observed after injection of saline.) MÜLLER's (1939) capillary-microscopical studies show

that intradermal injections cannot be given without injuring the capillary wall.

After 6 hours the actual tuberculin reaction sets in. Stasis occurs in the deep capillaries, the papillary and subpapillary capillaries dilatate.

The inflammatory flush spreads, an inflammatory exudate is formed which distends the tissues (formation of papillae).

The maximum effect is reached after 2 days.

Also BJØRNSTAD and BONNEVIER (1940) consider that the stratum subpapillare is the principal site of the Mantoux reaction.

ORIUCHI (1938) produced a Mantoux reaction in the skin of the axilla in 19 patients, excised that part of the skin, and examined it histologically. He found basophil granules in the tissue (Gewebsmastzellen) which, similarly as leucocyte cells in the blood (Blutmastzellen), may be antagonistic toward the injected tuberculin.

The histological and capillaroscopical investigations are complementary and the results agree with the common clinical observations. The results of capillary dilatation and inflammatory exudate are infiltration and flush, which are typical of the Mantoux reaction.

The responsibility of the skin for the tuberculin reaction

WORINGER (1928) and BIRKHAUG (1942) consider, on the basis of the literature and their own investigations, that tuberculin allergy is a function which is not connected with the immunity of the skin. CEPULIC and PINNER (1921) are of the same opinion, but BROKMAN and PROKOPOWICZ-WIERZBOWSKA (1926) maintain that the skin plays a passive role.

As the views of other authors differ as well, a further study of this problem was considered indicated.

The tuberculin sensitivity in different parts of the skin

Woringer and Sala (1929) performed tuberculin tests in children with tuberculosis of the glands and of the joints, simultaneously in different parts of the body, but using the same technique (Pirquet). The infiltrations were measured twice daily, and variations in the intensity and rate of development of reactions was observed.

The weakest reactions were found on the face and the insides of the hands, and the strongest on the trunk. As examples of the size of the tuberculin reactions the following may be cited:

Ventral		*Dorsal*	
Chest	17—11 mm.	Back	20—14 mm.
Thighs	9—9 »	Loins	18—10 »
Arms	9—9 »	Buttocks ..	14—14 »
Feet	6—6 »	Thighs	15—15 »
Cheeks	4—4 »	Calves	11—11 »

The reactions on the abdomen were almost the same size as on the chest, and on the forearm about the same as on the upper arm. As the injections were given segmentally no difference was in general observed between the ventral and the dorsal side.

Rackwitz (1934) made Pirquet tests on the trunk of tuberculous patients. In 52 per cent of the cases he found a stronger reaction on the chest and abdomen than on the arms and legs.

Studying 130 patients Ferria (1933) found that the skin on the chest and abdomen was more sensitive to Mantoux than the skin on the forearm. However, the difference was not invariable.

SALMONY (1921) made Pirquet tests in 21 patients on the skin of the chest and in two places on the forearm (5 cm. from the elbow joint and 5 cm. from the wrist).

Chest Pirquet	stronger than forearm Pirquet:	16 cases
» »	strongest of all arm Pirquets:	4 »
» »	less than upper arm Pirquet:	6 »

REICHEL and MILBRADT (1931) performed Mantoux tests in 4 patients and gave the following reaction results:

Age of patient	*Forearm*	*Chest*
61 years	6 mm.	17 mm.
75 »	7 »	12 »
70 »	12 »	18 »
63 »	10 »	16 »

WALLGREN (1924) examined 111 tuberculous children, using Hamburger's ointment test. In 57.8 per cent the reactions on the chest were stronger than on the flexor side of the forearm. OREL mentions the same occurrence in ***Handbuch der Kindertuberkulose*** (1930); this is probably based on WALLGREN's observations.

PIERRE-BOURGEOIS and BOQUET (1941) found that the reactions, performed symmetrically, varied in the different regions, being stronger in the deltoid than in the scapular region and the lower limbs.

In a previous work WASZ-HÖCKERT (1945) mentions having found in 276 healthy soldiers, 240 of whom were positive to Mantoux 1: 15,000, read after 72 hours, as follows:

	Stronger on the arm	*Equally strong*	*Stronger on the back*
Mantoux infiltration	25	28	187
Mantoux flush	4	46	190

The average of all Mantoux reactions, read after 72 hours, was 113 sq. mm. on the arm and 153 sq. mm. on the back.

Depth of skin

The depth of the skin is intimately connected with the question of the capillary density, i.e. the capillary meshwork is in general less close in deep skin and, as seen from the foregoing, the tuberculin reaction stronger.

The sole of the foot and the inside of the hand are exceptions as the skin is coarsest here (SCHISCHA 1900, SPALTEHOLTZ 1922) but the tuberculin sensitivity is weak in these parts (WORINGER and SALA 1929). However, as shown by MÜLLER and his school, these areas are subjected to strong mechanical irritation and due to this fact a greatly increased vascularization and an intensified circulation is present.

Density of lymph vessels and other local factors

The origination of the tuberculin reaction is undoubtedly the result of a complicated interaction between several different mechanisms acting locally and systemically. The capillary system, previously mentioned, is thus not the only factor although its role has been carefully examined in this investigation.

According to RACKWITZ (1934) the circulation of lymph plays an important part. The part taken by the lymphatic system has been specially studied by LAPORTE (1943, 1946) who found that B. tuberculosis, injected intravenously without being in contact with lymphatic vessels does not produce tuberculin allergy. BRANCH and CUFF (1930), and HUDACK and MCMASTER (1933) observed the significance of the lymphatic system.

The importance of humoral antibodies in the origination of allergy has been studied in stimulation and inhibition of the reticulo-endothelial system (RES.). A connection between the two has been shown by the use of several different methods. Inhibition of the RES. decreases tuberculin allergy and stimulation of RES. increases tuberculin allergy. In the experiments, blocking with dyes (Indian ink, Congo red, trypan blue) has been carried out, as well as splenectomy, X-ray radiation of the spleen, cholesterin diet etc. (KAWAGUTI 1938, LONGO and BORGHESE 1941, ALFÖLDY 1942, and a number of authors quoted by ANZALONE and PAGANO 1940.)

The importance of the cellular tissue has been studied with the aid of »spreading-factor» tests. Testicular extract, for instance, increases the resorption by the skin, with a resultant weakening of the tuberculin reaction (FIOCCO 1936), and the same results are obtained with hyaluronidase (ASBOE-HANSEN 1950).

A number of investigations have been done with vitamin C. The reactions are impeded by vitamin C, given by mouth or intravenously (HEISE, MARTIN and SCHWARTZ 1937, BIRKHAUG 1939, LONGO and BORGHESE 1941, CATTANEO and MORELLINI 1940, among others), but stronger reactions are obtained if vitamin C is injected together with tuberculin than if tuberculin is given alone (CEPULIC 1940 and NEGRONI 1944).

Factors in the anatomy of the skin which may affect the sensitivity to tuberculin

Depth of skin

SCHISCHA (1900) has shown that the epidermal thickness varies greatly in the different parts of the body:

	Depth in mm.	
Part of body	*Over the papillae*	*Between the papillae*
Sole of foot	0.525 —0.600	0.575 —0.625
Palm of hand	0.425 —0.500	0.473 —0.565
Lateral surfaces of fingers	0.160	0.200
Forehead	0.0210—0.0236	0.277 —0.0336
Below clavicle	0.0210—0.0378	0.0210—0.0504
Flexor side of forearm	0.020	0.0333
Flexor side of wrist	0.133	0.173

These figures are given by MÜLLER (1922 and 1937) in his comprehensive monographs on the capillary system.

The depth varies in the different individuals and due to differences in technique of measurement. SPALTEHOLTZ (1922), for instance, estimates that the depth in the sole of the foot is 2 mm., in the palm of the hand 1 mm., and in other parts of the skin 0.07 to 0.17 mm.

LODEMANN (1934) observed that the epidermis on the dorsal side of the body was deeper than in the corresponding regions on the ventral side. His estimations of the depth of the *corium* are

on the chest	2	mm.
» » back	4	»
» » sacral region	3	»
» » abdomen....	3	»

Capillary density

In studies of the anatomical and physiological conditions of the capillaries, capillaroscopy has been used in particular. After the introduction of this method by HUETER, in 1879, it has been further developed by LOMBARD (1912) and MÜLLER and his school. Furthermore, through microphotography permanent objective evidence and records have been obtained.

NESTEROW (1929), with the aid of these capillaroscopical examinations, estimates the capillary density as follows:

Hand and forearm	44	capillaries per sq.mm.
Shoulders	27	» »
Chest	23	» »
Forehead	25	» »
Cheeks	19	» »
Lips	46	» »
Thighs	29	» »
Calf and foot	41.3	» »

MÜLLER's results agree with NESTEROW's in that »die Akra stärker kapillarisiert sind als der Rumpf».

WENTZEL and ZOTTERMAN (1926) report the following results:

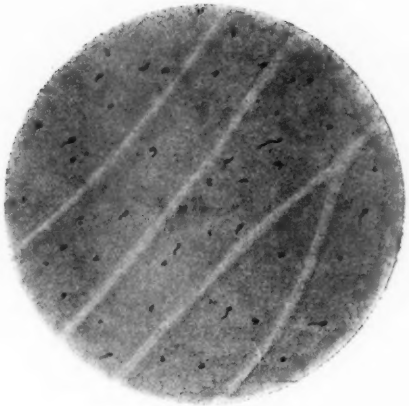
Back of hand	64	capillaries per sq.mm.
Forearm	47	» »
Cheeks	16	» »
Around mouth	20	» »

These capillaroscopical observations are thus in agreement.

LODEMANN (1934) performed histological serial sections and gave the following results:

Chest	50	papillae per ½ cm.
Back	40	» » »
Sacral region	32	» » »
Abdomen	30	» » »

MÜLLER (1922) gives capillaroscopical photographs of different regions, two of which are reproduced here. According to him the capillary density is analogous on the back and chest.



Normal skin

Extensor side of forearm
Magnif. 60

Normal skin

Chest
Magnif. 60

Density of lymph vessels

The density of lymph capillaries per visual field is about equal to that of the blood capillaries in the same area (HEWER 1947). Thus the density of lymph vessels is probably greater in the skin on the forearm than on the back.

Anatomical conditions in skin on back and forearm

The parts of the skin, subjected to investigation in this paper, the skin on the forearm and on the back, differ in anatomical structure, as seen from the above. It differs in depth, density of capillaries and lymph vessels. In the skin on the back there are not so many »organs» (sebaceous glands, hair follicles, sweat glands, muscles), LODEMANN (1934).

MÜLLER and his school have shown that vasomotor instability and psycho-somatic disturbances greatly affect the capillary findings in capillaroscopical examinations. But LENNARTZ (1921), HINTZE (1921), and especially CARRIER (1922) have shown that the capillary density in healthy people varies and that some capillary loops in the visual field are not filled. This occurs particularly in women (HINSELMANN HAUPT and NETTEKOVEN 1921). Mechanical irritation, stasis and temperature, dermographia, pressure, adrenalin, histamine, pituitrin, acetylcholine, nitrite and urethane (CARRIER 1922) affect the capillary status.

The dependence of the tuberculin sensitivity on other factors

Physical factors

SALMONY (1921) studied the influence of local hyperaemia and anaemia of the skin on the Pirquet reaction in tuberculous children. She induced local capillary hyperaemia with the aid of mustard paper, suction clock or hot sandbags. The Pirquet reaction was weaker than in the control tests, whereas with Bier's stasis the reaction was stronger. She also induced capillary anaemia with Esmarch's bandage and intracutaneous injection of adrenalin. The Pirquet reaction was considerably stronger. In stasis tests CIMMINO (1928) obtained the same results as SALMONY. But, in venous hyperaemia (stasis) REICHEL and MILBRADT (1931) observed weaker reactions. They got stronger reactions in hyperaemia tests (bath).

SEEBERG (1947) studied the effect of freezing the skin with carbon dioxide on the tuberculous allergy in 13 patients and found milder reactions to different dilutions in all the patients. According to BING, CARLSTEN and CHRISTIANSEN (1945) capillary dilatation is caused by freezing.

Radiation equivalents

GENOESE (1927), TALON-CHAUVEAU, VALTIS and TALON (1927), MEDOWIKOFF (1930) and SHINDO (1938) found reduced sensitivity to tuberculin in skin exposed to sun-rays. MICHAILOWSKIJ (1927) observed no difference, but on an interval of some considerable time, after exposure to sunlight, the reactions were stronger. SEEBERG (1947), with careful technique, found distinctly weaker reactions in sunburnt skin.

A number of investigators, some of which are surveyed by SEEBERG, have shown that ultra violet radiation diminishes the reaction to tuberculin, even complete anergy may be observed. SEEBERG's own investigations verify the former observations.

Roentgen radiation of the skin increases, on the other hand, the tuberculin reaction (ABRAMOWITZ and RABUCHIN 1928, BONNANO 1929, CONRAD 1932, FUNDARO 1937).

Drugs and hormones acting on the autonomic nervous system

In their summarising article ANZALONE and PAGANO (1940) — with good reason — state that this part of the tuberculin-allergy is incompletely studied, and the results do not justify far-reaching conclusions.

The question will however be reviewed briefly, as a number of the preparations are capillary regulating, and as the most recent investigations into rheumatoid conditions seem to approach the hormonal question. Allergy may largely be connected with, or dependent on the autonomic nervous system — this is an opinion voiced by CEPULIC (1940).

Adrenalin, the effect of which is vasoconstrictive, has also been tested with regard to practical tuberculin diagnostic work. On addition of adrenalin to tuberculin, stronger reactions have been observed by BOUVEYRON (1921, 1932), BENTIVOGLIO (1931), PLATONOW (1935), KLOSTER (1935), HANSEN (1937), BJØRNSTAD and BONNEVIER (1940) and CEPULIC (1940), among others. HANSEN's, and particularly BJØRNSTAD and BONNEVIER's investigations are so comprehensive that the significance of indefinite or contradictory results is decreased, as mentioned. Addition of adrenalin for the purpose of increasing the effect was suggested at the B.C.G. meeting in Copenhagen, in 1949 (USTVEDT), for Pirquet tests. Ephetonin and epinephrine, with analogous effects to adrenalin, do not affect tuberculin allergy to any extent when given parenterally (MARIANI 1941, and MARIANI and INGRARO 1941).

Atropine, with vasodilatative effect, injected parenterally, increases the sensitivity to tuberculin (MARIANI 1941, and MARIANI

and INGRARO 1941), as do morphia and caffeine (PERMJAKOW 1926).

GUIOT and GHALI (1941) observed diminished tuberculin allergy on blocking of the sympathetic.

Hormones, in general, seem to stimulate or increase the tuberculin sensitivity. Illustrative examples are given by CEPULIC (1940), who studied the tuberculin allergy in persons with thyrotoxicosis and diabetes in whom the tuberculin reaction was positive or stronger after injection of hypophysin or insulin. Extract of hormone secreting glands and pure hormone preparations such as thyroxin, thyroidin, hypophysin, and sex hormones increase the tuberculin allergy (PLATONOW 1935, SYLLA 1939, CEPULIĆ 1940, EDILIO 1941). The effect of thyroid extract is particularly marked in this respect. Animal tests with thyroidectomy or supply of thyroidea point in the same direction (SCHITTENHELM and BAUER 1935, VIETHEN 1936, LUCACER 1940, LENTINI and SPINA 1940). This is due to the influence of the thyroid gland on the ability of the skin to react, according to LENTINI and SPINA (1940).

Three groups of guinea-pigs were tuberculin tested by LENTINI and SPINA: 1) castrated animals, 2) animals given corpus luteum and 3) animals given folliculin. The castrated animals reacted strongly, as did the second group, but in the third group the reaction was weaker than in the others.

The female sexual cycle probably influences the tuberculin allergy. During the first days of menstruation the tuberculin allergy is weaker (CONFORTO 1937), and during the last stage of pregnancy, as well as during the first days of puerperium the allergy is considered to be less (STERN 1910, BAR and DEVRAIGNE 1911, NOBECOURT and PARAF 1920, PARISH 1938, and others). These results have not been verified in recent investigations, however (CONN 1942 and LICHTENSTEIN 1942). No difference was found in pregnant or non-pregnant animals in tests performed by INGIULLA and DAVOLI (1941) and WADE (1942). This question is still unsolved, according to RICH (1946).

It has been mentioned that constitutional factors affect the size of the reactions (KLARE 1935, SEIFFERT 1938, ICKERT 1940 and HOMMEYER-BRENTANO 1946). The sensitivity is also changed in schizophrenic patients (BLOMQVIST 1940).

Owing to its central place in the chemical physiology of the reactions of the skin, the relation of *histamine* to tuberculin has been closely investigated. ROBERTS, in an unpublished microscopical work, which has been cited in *Macleod's Physiology*, observed that histamine, the physiological effect of which has been principally clarified by LEWIS (1927), increased and dilatated the capillary loops from 25 visible loops to 70. The effect of histamine weakens the tuberculin reaction (BRETON 1943, EULER 1947, and others).

Other general factors

Some other factors, in addition to those already mentioned, affect the tuberculin allergy considerably, and must now be treated.

At the XII meeting of the Nordiska Tuberkulosläkarföreningen in Stockholm, in 1941, LEMMING, discussing the importance of negative tuberculin reactions suggested the following classification:

1. Absolute anergy
   - In an individual who is not, and has not been infected with tuberculosis.
   - Anergy in the stage of incubation.
2. Relative anergy
   - A. Temporary anergy
     1. in connection with certain infections
     2. after tuberculin treatment of long duration (BIRKHAUG: iathergy).
   - B. Negative anergy
     1. in advanced forms of pulmonary and miliary tuberculosis and tuberculous meningitis.
   - C. Positive anergy (HAYEK)
     1. in active tuberculosis without cachexia
     2. in clinically healed tuberculosis (especially age-anergy).
   - D. Positive, specific anergy (JADASSOHN) in morbus Schaumann?

The table is instructive and is given in full as certain points of connection are found with the following.

Various tuberculins and tuberculin tests

Comparison of the results of tuberculin tests performed by various authors is rendered difficult, due to the methods and criteria for positive reactions being inadequately described, and also owing to the use of varying tuberculins. Tuberculin tests in the same individual with different tuberculins and methods naturally give different reactions.

Koch's old tuberculin is still in general use, but as it is produced in different laboratories all over the world, its action is not the same. Since the days of Koch some new tuberculins have been produced, particularly in Italy, South America, Germany and Japan. American investigators, with a view to eliminating unspecific protein components in the old tuberculin, after lengthy investigations, found a purified protein derivative, PPD. Florence Seibert's first report on PPD. was published in 1934. Since then she, together with her co-workers, have proceded assiduously and in 1948 Seibert stated that she had arrived at a »more specific diagnostic reagent», and therewith ruled out pseudoreactions. Another new tuberculin, frequently mentioned in the American literature and introduced by Corper (1941) is called »autolytic tuberculin» and is used in particular in the »transdermal or transcutaneous tuberculin test» (TTT).

In addition to the classical Pirquet, Mantoux, and Moro tests, some other tuberculin tests have been adopted, but in the case of theoretical, exact examinations, the Mantoux technique with different dilutions seems to be the most reliable.

In assessing the literature on tuberculin the factors (different methods, different tuberculins) should thus be closely observed. This has been particularly stressed by Holm (1934) who in his well-known work goes thoroughly into the question of standardization. During later years international standardization of tuberculin has been discussed (WHO, 1948), also technical questions about tuberculin (European BCG-meeting, Copenhagen 1949).

Different stages of tuberculosis

Since ELLERMANN and ERLANDSEN (1909) and PIRQUET (1914) introduced graded, quantitative tuberculin tests in patients with tuberculosis in different stages, a number of investigators have studied a large series for the purpose of determining »allergometrically» the activity of the process by tuberculin titration. In the literature opinions differ regarding the value of such examinations. It can now be definitely said that a certain clinical-pathological process does not correspond mathematically to a certain tuberculin allergy. The tuberculin allergy varies during the course of disease, as numerous investigations show.

The »focus theory», i.e. stronger allergy, closer to the tuberculous focus than further away — has not been proved to be correct in extensive investigations (SANTI 1933, FERRIA 1933, HERTZBERG 1934, among others).

Different ages, epidemiological and social factors

The age distribution influences the tuberculin sensitivity; this is probably connected with different liability to infection and morbidity. The tuberculin positive curves from different countries show, however, largely similar traits.

Epidemiological factors play a part as the tuberculous infectivity percentage varies in different countries and in different parts of the same country. The percentage always differs in towns and rural district. SAVONEN's (1937) investigation of these questions seems to be the largest.

The figures giving the incidence of tuberculin positive reactions in different classes of society, living within the same area, differ as well. Children from the slums and from well-to-do homes show quite different tuberculin figures, which is seen from the literature.

Geographical and social factors may be related to nutritional conditions. The effect of the composition of the food on tuberculin sensitivity has been investigated by BONNANO (1937), TOURBINA (1940), and a diet of carbohydrates has been found to produce a strong tuberculin allergy, and a fatty diet a weak tuberculin allergy, and that the alkalic-acid balance plays a part.

A group of the population which everywhere shows a characteristic tuberculin reaction comprises people in presenium and senium. Investigations into this question have been carried out in several quarters (MÜLLER-DEHAM and JOKL 1929, REICHEL and MILBRADT 1931, HERLITZ 1939, LEMMING 1941, COSTE, BERNARD and HERVAT 1941, COURY and MARLAND 1948, and in Finland ANTTONEN 1941). The cause of anergy and dysergy to tuberculin in the aged has been explained partly by the tuberculous processes in these being completely healed (LEMMING) or by a generally reduced ability to react in the atrophied skin (MÜLLER, DEHAM and JOKL).

Tuberculous or other cachectic conditions, physical exhaustion

Already PIRQUET (1908) and MANTOUX (1910) observed that the tuberculin allergy in cachectic patients, during the most difficult final stages of tuberculosis, was reduced or no longer present. The same circumstance has been observed in moribound or cachectic patients with non-tuberculous etiology (e.g. cancer). Transient anergy or disallergy has been observed after great physical exhaustion in muscular strain (MARTINI-DUBOUSQUET and GROLLET 1941).

MUSACCHIO (1940), BROSIUS and WOODRUFF (1944), and WOODRUFF 1946 and 1947) report histopathological findings on examination of severely ill tuberculous patients who, immediately before death, were anergic, and found many acidfast bacilli in the sections and also in the sputa of anergic tuberculous patients, whereas the sputa of the super-allergic contained only a few bacilli (WOODRUFF).

Seasonal and daily variations

It has been said that there is a seasonal variation in the tuberculin sensitivity, the allergy being greater from December to July, (HAMBURGER 1920, and PEYRER 1921). Variations at different times of the year were found by HAAHTI (1933) in a large number of cases, but he was not inclined to call them seasonal variations. SAVOLIN (1934) mentions similar occurrences. In a very instructive report, HOWE (1938) shows daily irregularities in connection with »daily diastolic blood-pressure levels».

Racial factors

In the investigations reported from the USA, where the cases examined often comprise individuals from different racial groups, variations have been observed. DAHLSTROM (1940) found that the tuberculin sensitivity of Italians and Negroes was lower than in other races.

Certain diseases and drugs

Since PIRQUET (1908), and MANTOUX (1910), reported that tuberculin allergy becomes weaker in measles similar observations have been made by various investigators. According to the reports measles, whooping cough, German measles, scarlatina, paratyphus, diphtheria, influenza and malaria reduce the tuberculin sensitivity. Furthermore, weak allergy is described in diseases with malignant tumours, gastric ulcer, and syphilis, and increased allergy after surgical operations, in diabetes and goitre. The anergy in Boeck's sarcoid has been closely studied.

The action of certain hormones and drugs on the tuberculin allergy has already been mentioned. Quinine (VERGER 1941), cysteine and sodiumthiosulphate (CATTANEO and MORELLINI 1940) given by mouth decrease the allergy. Sulphonamides (PÄTIÄLÄ 1943, SCHUPPLI 1945) and streptomycin (DEBRÉ et al. 1948) decrease the sensitivity as well.

The influence of rheumatic infection on the tuberculin sensitivity

In quantitative tuberculin examinations ROSENBERG (1913) tested rheumatics, among others, and found as follows:

	Positive to 0.1 cc. Mantoux injection of old tuberculin dilution			
	1: 5,000 (1/50,000 mg.)	1: 10,000 (1/100,000 mg.)	1: 50,000 (1/500,000 mg.)	1: 100,000 (1/1,000,000 mg.)
Rheumatic cases (Non-tuberculous)	95.0 %	86.7 %	77.8 %	60.0 %
Number of cases	(17)	(13)	(14)	(3)
Control cases (Non-tuberculous, non-rheumatic)	51.0 %	31.3 %	6.1 %	14.3 %

On the basis of these figures ROSENBERG considers that the tuberculin allergy in patients with rheumatic diathesis is higher. Among »rheumatics» he includes: angina follicularis, arthritis acuta and chronica, and »Muskelrheumatismus». ROSENBERG is quoted in CITRON's manual (1923).

CHIARI and MATRICARDI (1938) tested 295 children between the ages of 3 and 14 (rheumatic fever, endocarditis and chorea minor) and found that 42.4 per cent reacted positively to tests with up to 1.0 mg. Mantoux. The tuberculin negative (170 children, 57.6 per cent) retained their anergy for several years. Only 2 out of 70 children with exudative pleurisy were negative to 1.0 mg. Mantoux.

ONTANEDA, PASQUALINI and FERLONI (1938) tuberculin tested 40 adult patients with rheumatic fever. Twenty-three of these were tuberculin positive. The percentage was the same in persons without rheumatic affections.

PELLEGRINI and CARINCI (1939) obtained in 21 patients suffering from rheumatic fever 7 negative cutaneous reactions (Pirquet) and 3 negative intradermal reactions.

In 2 cases out of 20 with rheumatoid arthritis LAI and JORIO (1940) obtained positive reactions to anatuberculin and 18 negative reactions.

CARINCI and RE (1940) performed Mantoux tests in 32 patients with polyarthritis:

			Positive to Mantoux	
20	12	polyarthritis acuta	8	
	8	» subacuta	6	14 cases
12	5	» ac. and tub. activa	5	
	7	» » » » inactiva	6	11 cases
32				25 (78 %)

According to these authors the tuberculin reactions are more often positive in patients with polyarthritis than in other, non-tuberculous, groups of disease.

CANETTI and LACAZE (1940) found the following allergy series in 58 patients with rheumatoid arthritis:

Mantoux 1:1,000 reaction diameter read in mm.				1: 100 + 1: 1,000 −	1: 100 −
25	20−25	15−20	10−15		
3	10	20	16	2	7
Total 58					

Seven out of the 58 patients examined were thus completely tuberculin negative.

In their work on tuberculin allergy in »malattia reumatica» BONCOMPAGNI and TURCHINI (1946) summarize the results in the following table:

Diagnosis	*Cases*	*Different tuberculins*	*Allergic*		*Anergic*		*Allergy*		
							small	*medium*	*great*
Polyarthr. acuta	21	TB	15	71 %	6	29 %	4	4	7
		TU	12	57 %	9	43 %	7	4	1
Polyarthr. subacuta	14	TB	13	93 %	1	7 %	2	4	7
		TU	12	86 %	2	14 %	5	2	5
Polyarthr. chronica	100	TB	89	89 %	11	11 %	14	37	38
		TU	74	74 %	26	26 %	30	32	12

Hence they considered that the tuberculin allergy in their cases with chronic polyarthritis was higher than in the cases with acute polyarthritis.

EDSTRÖM, in 1943, collocated the results of tuberculin tests from a large number of patients in hospital in Lund for the years 1938—42, consisting of acute and chronic polyarthritis. They were first tested with Pirquet, and if the result of the test was negative, Mantoux 1.0 mg. was given. The ages varied from 6 to 55 years.

	Polyarthr. acuta	*Polyarthr. chronica*
Tuberculin positive	204 (66 %)	730 (80 %)
Tuberculin negative	93 (30 %)	168 (18 %)
Uncertain (Pirquet and Mantoux 1.0:? or not performed)..	11 (4 %)	20 (2 %)
	308	918

Edström, as well as Boncompagni and Turchini, found higher tuberculin positivity among the chronic polyarthritics than among the acute. He points out, however, that the patients with chronic disease were older. He compares the tuberculin positive reaction of the rheumatic patients with 2,167 other, non-rheumatic patients from the same clinic and finds good agreement in the two groups. But he assumes that if any differences were to be found, the tuberculin positive reactions in the second group (healthy) would be slightly higher. He has furthermore found cases in which the rheumatic patients were hyperergic.

Kahlmeter (1934) reports that he has observed strong, positive reactions with Mantoux tuberculin 1:1,000 and 1:10,000, and in some cases even with 1:100,000 and 1:1,000,000, an occurrence which he has never found in any other disease.

In a discussion at a congress, Ylppö (1930) mentioned that tuberculin tests in children with rheumatic fever are frequently strongly positive. But, on the other hand, »bei chronischer deformierter Arthritis (ist) die Tuberkulinreaktion in der Mehrzahl der Fälle negativ, was wohl darauf zurückzuführen ist, dass wir hier immunobiologisch mit ganz besonderen Kindern zu tun haben». In his lectures Ylppö states that he has found cases in which children with rheumatoid arthritis, in spite of unmistakable clinical signs of tuberculosis, have remained constantly tuberculin negative. Helsingius and Ylppö (1925) give a numerical report in their work. The children described in the table below belong to a milieu in which tuberculosis infection is frequent.

Diagnosis	*Pirquet +*	*Pirquet −*	*Total*
Rheumatoid arthritis	3 (14.3 %)	19	21
Rheumatic fever	19 (65.5 %)	10	29
Chorea minor	36 (66.7 %)	18	54

The cases in the different investigations referred to vary greatly as regards stage of disease, age of patient and tuberculin technique. A tendency is however observed on combining the examinations:

Author	*Number of cases*	*Tuberculin positive*		*Notes*
		cases	%	
Rosenberg	17	16	95 %	
Chiari and Matricardi	295	125	42 »	Polyarthr. acuta
Ontaneda et al.	40	23	57 »	»
Pellegrini and Carinci ..	21	18	85 »	»
Carinci and Re	20	14	70 »	»
Boncompagni and Turchini	35	29	80 »	»
Edström	308	204	66 »	»
	736	429	60 %	
Canetti and Lacaze	58	51	88 %	Polyarthr. chronica
Boncompagni and Turchini	100	89	89 »	»
Edström	918	730	80 »	»
	1,076	870	81 %	

In these 1,812 cases of rheumatic affections of the joints 60 per cent of the acute cases and 80 per cent of the chronic cases reacted positively to the tuberculin test. Edström's investigations, which are much more extensive than those of the other authors, greatly influence the result which is in accordance with the general tendency. Ylppö is the only author who is of quite contradictory opinion.

Furthermore, it is observed that some authors (Edström, Canetti, and Boncompagni) have found highly allergic reactions. The opinions differ somewhat regarding the rheumatic cases, whether their tuberculin sensitivity is higher than in persons without rheumatic affections. Edström's investigations, in particular, support the conception of the sensitivity being about equal.

Lai and Jorio's work is not included as they used a weaker tuberculin test (ointment?).

Reitter and Löwenstein (1932) mention, regarding their cases of rheumatic fever, that they are »insbesondere ganz ausserordentlich tuberkulinempfindlich».

The problem

Such a variety of different mechanisms take a part in the origination of tuberculosis reactions that a clear and simple explanation of the occurrence cannot be given on the basis of the present scientific knowledge. The majority of viewpoints have, no doubt, been observed and mentioned in the extensive literature on tuberculin, but their interrelationship and role have not been clarified.

The skin itself and its anatomical characteristics represent only some of the factors, but its role has, however, been considered important enough to merit a further explanation.

The information in the literature differs, regarding the important rheumatic group of disease, of such topical interest.

The purpose of this investigation is to study:

1) The influence of the anatomy of the skin on tuberculin sensitivity, when
   a) Mantoux test is done in analogous parts of the skin,
   b) Mantoux test is done in parts of the skin with slight anatomical differences,
   c) Mantoux test is done in parts of the skin with great anatomical differences.

2) The tuberculin allergy in patients with rheumatoid arthritis. The following were examined for comparison:
   a) healthy people
   b) tuberculous patients who have not had rheumatic diseases.

3) Do common secondary infections affect the tuberculin allergy in healthy people, rheumatoid arthritics, and tuberculous patients?

The author's own investigations

I. The Mantoux reaction in different parts of the skin

Material

In order to study the tuberculin sensitivity on parts of the skin with different anatomical structure a series of 235 tuberculous patients was examined. These patients were treated at the City of Helsingfors Hospital for Tuberculous diseases, in either the Department of the University or of the Commune. Part of the child material, 42 children, is from the Kiljava Sanatorium.

Age and sex distribution of the cases examined is given in the table:

	Adults						
	15—19	20—29	30—39	40—49	50—59	60—69	15—69
Men	—	10	18	34	6	8	76
Women	7	18	12	9	3	1	50
Total	7	28	30	43	9	9	*126*

Children				
0—2	3—5	6—10	11—15	0—15
30	28	34	17	*109*

Weak, cachectic and febrile patients were not included in these studies. The adults were patients with pulmonary tuberculosis, whose processes were improving clinically and who were allowed to be up and to rest in the sleeping-loggia. The child material was homogeneous in that all were patients with primary tuberculosis with roentgenological swelling of the hili. No cases of miliary tuberculosis or tuberculous meningitis were thus examined, nor were cases with primary tuberculosis in the initial febrile stage included.

Technique

The tuberculin tests were performed with Koch's old tuberculin (Behringwerke) in two solutions, 1: 10,000 and 1: 100,000. Sterile saline solution was used as the dilution fluid. The tuberculin solution 0.1 cc. was injected intracutaneously, the stronger Mantoux injection thus containing 0.01 mg. and the weaker 0.001 mg. old tuberculin.

Fresh tuberculin solutions were prepared for each occasion when tests were made and the dilution was performed with dry-sterilized clean pipettes of the size of 0.1, 1.0 and 10 cc. No preservative fluid (phenol, etc.) was used in order to avoid non-specific pseudo-reactions.

0.5 cc. syringes graduated for 0.01 cc. were used for the Mantoux injections, separate syringes were used for each solution, and also for the control tests with saline solution. Needles No. 18—20 with short bevel and platinum cannula were carefully fitted to the syringes so as to prevent any fluid escaping during the injection.

The Mantoux tests were done 1) on the extensor side of the forearm (dorsal side), midway between the elbow and the wrist, and 2) on the back, between the shoulderblades, one or two cm. from the mid-line. The skin was cleaned before the injection with ether and in exceptional cases with alcohol, instead of ether — or in addition to ether — but in these cases the skin was allowed to dry before the injection. Paretsky (1936) has observed that the Mantoux reactions are weaker if the injection is made before the skin has dried, when alcohol has been used to clean.

Great care is exerted regarding the technique of injection. At an early date the author learnt to rely upon the following procedure, which is recommended by WALLGREN (1935) as well: the skin is lifted into a fold (Falte) and the injection needle is inserted almost parallel with the skin, the bevel of the needle turned upward. By careful rotation of the syringe the needle penetrates intracutaneously into the right layer subepidermically, not too superficially, not too deeply, and definitely not subcutaneously. The needle is inserted 3 to 4 mm. into the skin, the bevel being kept visible through the cutis all the time. The tuberculin is injected slowly and carefully, the graduation on the syringe and the wheal being watched. After a moment's pause the needle is withdrawn (HOLM 1934), a sterile cottonwool swab is pressed on the site of injection to prevent the tuberculin from escaping when the needle is withdrawn.

Reading of the reaction

The Mantoux reactions were read in daylight 24, 48 and 72 hours after the injection.

The Mantoux reaction consists of 2 elements: *erythema* and *infiltration*. HOLM considers that the erythema is caused by a local vascular reaction and that the infiltration is caused by reaction of the cells. The two components do not follow one another during the different phases of the reaction. The erythema appears already during the first twenty-four hours of reaction — often suddenly flaring up — but on the second to third day it generally dies down in the part of skin which surrounds the infiltration and persists for a day or two in the raised, infiltrated region of the skin. The colour gradually fades and 4—6—8 days after the injection the infiltration is almost the colour of the skin, which also fades gradually. Two to four weeks later the place for the Mantoux reaction is marked by a pigment spot. These two components are considered separate processes of the tuberculin reaction and seem to be fairly independent of one another. Of these the erythema is the more variable and may at times be absent in quite distinct Mantoux reactions with infiltration of 10 to 15 mm. HOLM has studied this phenomenon closely and has illustrated his observations graphic-

ally. In observations of intracutaneous tuberculin reactions it has often been considered — particularly in more theoretical examinations — that both components should be noted separately.

The area of *erythema* varies from day to day in the same patient. Its size is however similar to the area of the infiltration. In the present investigation, in which a comparison of the strength of the reactions is the essential object, the author has studied erythema as a colour phenomenon only, and has not considered its size. This has been done by using T. W. Tallqvist's haemoglobin colour-scale, which was formerly commonly used. Assessment according to this colour-scale should be fairly objective, in the author's opinion, as lighting conditions were always similar (daylight) and as the colour reactions were always evaluated by the author himself. Colour No. 20 in this colour-scale corresponds to the colour of the skin, or a very weak reaction, and No. 50 to the strongest reactions. Variations are designated + and — in the records. As mentioned in a previous work (WASZ-HÖCKERT 1945), the shades of the different colours are not quite identical to those in the colour-scale, but they are easily divided into categories on the basis of their intensity of colour.

The most important component of the Mantoux reaction is the *infiltration*. A diameter (for instance 10 mm.) or the length of two perpendicular diameters (for instance 10 mm. × 10 mm.) are used as the measure for this component. In the latter case the size of the reaction is generally given by the mean of the diameters (in mm.) or by their product (in sq.mm.). The Mantoux infiltrations are often elliptical. NEGRONI (1944) suggests therefore that the reactions can be expressed mathematically by the formula

$$\frac{\pi D d}{4}$$

in which D is equal to the greatest diameter and d equal to the smallest diameter. In works in which GRÖER's (1934) investigations have been re-examined, PETRANYI and MOTIKA (1936) point out that an objective measure of the reactions is obtained by measuring the diameters only in the case of the reactions being oval or round. They illustrate their statement by graphs and suggest that a more detailed method should be used.

PETRANYI and MOTIKA marked the Mantoux reaction with staining solution and traced it on to cellophane paper. The figure was then copied on to Kimographion paper, cut out and weighed. As the specific gravity of the paper was known the exact surface of the reaction infiltration was obtained directly. With this method they obtained results which were completely different from those obtained with the commonly used diameter measuring method. The material consisted of 288 children, and by measuring, positive reactions were obtained in 11.9 per cent, and by weighing in 50.7 per cent. Such exact methods, although probably time-consuming, have been suggested by other investigators as well. STEWART (1933) traces the reactions on celluloid paper, cuts them out and weighs them: i.e the same method as PETRANYI used. The method was modified by HAAHTI (1943) in that he copied the figures from cellophane paper on to millimetre paper and calculated the surface directly in sq.mm. LEVADITI (1918) used planimeter, so did SEEBERG (1947) in his dissertation. RIST (1941) and BERNARD and LOTTE (1946) have suggested more complicated methods.

In the first section of this work the following modification by PETRANYI has been used: The border of the infiltration, which is determined by means of inspection and palpation, is marked by a thin line drawn in ink. The figure is copied on to a cellophane paper which is pressed against the Mantoux reaction. The cellophane figure is transferred with tracing-paper on to a square index card, cut out accurately and weighed on a chemical balance, to within 0.1 mg. After determining the specific gravity of the card the size of the surface is calculated and noted to within 0.1 sq.mm.

In the second section of this work this time-consuming procedure was abandoned. Instead, the greatest diameter and the diameter at right angles to it were measured with a transparent celluloid ruler. On close study of the two methods it was found that the required accuracy was obtained by the simpler method.

The technical error in Mantoux reactions

The size of the reaction surface P_i, obtained in repeated observations of the same person, is not, of course, a uniformly determined function of his tuberculin sensitivity. It is liable to occasional

fluctuations caused mainly by biological variations, and also by differences in the technique of the injection. The size P_i is, in other words, a »random variable». The standard deviation will be designated $\sigma_1(P_i)$. Furthermore, it is assumed that on measuring the quantity P_i, its true value is not obtained, but a value R_i, in which is included an error of measurement. The measured value R_i may thus be expressed by the formula

$$R_i = P_i + Z_i \tag{1}$$

in which Z_i is the error of measurement. It is assumed that the error of measurement Z_i is a normally distributed »random variable» which is independent of P_i and whose mean is 0 and dispersion $\sigma_2(Z_i)$. The standard deviation $\sigma(R_i)$ of R_i, which in the following will be termed the *»error of technique»*, is consequently obtained from the equation

$$\sigma^2 = \sigma_1^2 + \sigma_2^2 \tag{2}$$

The size of σ_2 (as well as σ, to the part caused by differences in giving the injection) is naturally greatly dependent on the skill and accuracy of the examiner and on the methods of measuring. In this connection it should be pointed out that even a fairly large standard deviation σ_2 in the error of measurement may be allowed without rendering impossible the use of the standard deviation σ in the result of measurement as an approximation of the standard deviation σ in the reaction surfaces. From the equation (2) follows

$$\sigma_1 = \sigma \sqrt{1 - \left(\frac{\sigma_2}{\sigma}\right)^2} \tag{3}$$

Holm, who is probably the only investigator who has calculated the standard deviation σ in intracutaneous tuberculin reactions in man, obtains the approximative value $\sigma = 20$ per cent. If the mean R_i of the size of the measured reaction surfaces is 200 sq.mm. and their standard deviation is carefully assumed to be only 13 per cent or 26 sq.mm., and the standard error in the measurements is estimated to be 10 sq.mm. which is used in an estimation of σ_2, it is found, according to equation (3) that σ_1 is 24 sq.mm. The standard deviation of the measured reaction surfaces is thus only

about 8 per cent greater than the standard deviation of the true reaction surfaces.

In order to obtain an estimation of the technical error σ (R_i) in the present work the author, in 1946, carried out *double determinations of Mantoux in 82 adult tuberculous patients.* These double determinations were performed as follows: In completely analogous parts of the skin, on either side of the mid-line of the back, and exactly at the same distance from it, interscapularly, two Mantoux injections of 0.1 cc. tuberculin solution 1: 10,000 were given (i.e. 0.01 mg. old tuberculin). The reactions were read 24, 48 and 72 hours later. Thus 82 samples were obtained at each reading, each comprising two values for R_i, the area of the reaction surfaces in tuberculous patients. All these measurements have the same standard deviation σ (R_i), the technical error, as the mentioned error may of course be assumed to be independent of the subject examined. The last-mentioned circumstance makes it possible to estimate the standard deviation σ (R_i) from these 82 samples.

R is the designation for the Mantoux reaction on the left of the mid-line, which was done first. R_k is the designation for the control reaction on the right.

TABLE 1

Double determinations with Mantoux 0.01 mg. given between the scapulae in analogous parts of the skin.

	24 hours		48 hours		72 hours	
	R	R_k	R	R_k	R	R_k
47 men, mean in sq.mm.	210.9	211.4	207.9	212.5	206.0	207.1
35 women, » » »	214.6	207.3	235.3	229.2	216.3	216.9
82 patients, » » »	212.5	209.7	219.6	219.6	210.4	211.3

The following expression was used in the statistical treatment (CRAMÉR 1949):

$$\sigma = \sqrt{\frac{\Sigma (\lg R - \lg R_k)^2}{2n}} \qquad (4)$$

σ is the deviation if *normal logarithmic* distribution is assumed. $\lg R - \lg R_k$ is the difference between the logarithms for the surfaces in the reaction pairs. The number of reaction pairs is designated n.

TABLE 2

The material treated according to expression (4).

	24 hours	48 hours	72 hours
Men	0.0452	0.0529	0.0579
Women	0.0588	0.0513	0.0622
Total	0.0514	0.0522	0.0597

It is observed that the distribution for the reaction surfaces differs by 11 to 15 per cent from the mean value of the size of the reaction surfaces in the same person.

According to the table the distribution varies between 11 and 15 per cent. This figure lies within the borders of what is considered typical of biologic reactions (20 per cent). The result thus verifies HOLM's assumption.

TABLE 3

The anti-logarithms for the values in Table 2.

	24 hours	48 hours	72 hours
Men	1.11	1.13	1.14
Women	1.15	1.13	1.15
Total	1.13	1.13	1.15

For the purpose of deciding if the two methods for reading the reactions, used in this work, give the same results, *the copy-weighing method as well as the ordinary measurement of diameters was used in the reading of 154 Mantoux reactions.* The material for this investigation includes reactions of all sizes. As two terms for expressing the same Mantoux reaction are used (for instance: 12 mm. $\times$ 10 mm. and 117.5 sq.mm.) it is possible to determine whether the two methods give the same results.

The method for measuring with diameters will be termed *A*, and the more exact method *B*. The surfaces measured according to method *A* will be designated *a* and those measured according to *B* will be designated *b* and the mean of the reactions *m*. We thus have:

a = the product of the diameters, measured perpendicularly to each other.

b = the surface in sq.mm. according to the more accurate method.

Surface	Number of readings	$m \pm \varepsilon$ sq.mm.
a	154	207.6 ± 7.41
b	154	194.9 ± 7.70

The table shows that the difference between the means of the surfaces *a* and *b* are not statistically significant. This agrees with the consideration of equation (3) and a natural explanation is obtained in the circumstance that the standard deviation in the surface, caused by biological variations, is relatively great.

In a large material with reactions of all sizes, methods *A* and *B* thus give the same result. For the purpose of obtaining a connection between the different results, *a* and *b*, in measurement of the same reaction, *b* is assumed to be linearly dependent on *a*, and the regression line is calculated:

$$b - m_b = k\,(a - m_a)$$

$$m_a = \frac{1}{n}\sum a_i \qquad m_b = \frac{1}{n}\sum b_i$$

$$k = \frac{\Sigma\,(a_i - m_a)\,(b_i - m_b)}{\Sigma\,(a_i - m_a)^2}$$

The values a_i and b_i represent the size of the separate surfaces and n the number of surfaces observed. The following is obtained

(5) $$b = 0.917\ a - 4.6$$

The mean errors for m_a and m_b are obtained according to the formula

$$\varepsilon = \sqrt{\frac{\Sigma\ (x_i - m_x)^2}{n\,(n-1)}} \tag{6}$$

The mean error for m is obtained according to the formula

$$\sigma^2_m = \frac{\Sigma\ (b_i - m_b)^2}{(n-2)\,\Sigma\,(a_i - m_a)^2}$$

Taking these mean errors into consideration (5) may be written:

$$b = (0.917 \pm 0.040)\,a - (4.6 \pm 10.5) \tag{7}$$

The approximative result is thus

$$b = 0.92\,a \tag{8}$$

The error in (8) in a reaction of, for instance, 200 sq.mm. is on an average 20 sq.mm. according to (7). The relation (8) is thus sufficiently accurate for the purpose in question.

Calculating the correlation coefficient r according to the formula

$$r^2 = \frac{\left[\Sigma\ (a_i - m_a)\ (b_i - m_b)\right]^2}{\Sigma\ (a_i - m_a)^2 \cdot \Sigma\,(b_i - m_b)^2}$$

$r = 0.88$ is obtained, which, as expected, shows that the standard deviation around the straight lines (8) is comparatively small.

It seems surprising that b and a are almost of the same size. If a reaction is c mm. $\times\, d$ mm. its surface might be expected to be of the size $\frac{1}{4}\,\pi\ c\ d$ which is the case when the reactions are elliptical in shape. In other words, instead of (8) the relation

$$b = 0.79\,a$$

would be expected.

That this is not the case is partly due to the shape of the reactions, which may be very irregular, and also to the weighing method used in this investigation being very sensitive to systematical errors.

In order to verify this assumption, *50 reaction figures traced on to cellophane paper have later been measured by a planimeter.*

The surfaces according to the weighing method b
The surfaces according to the planimeter method c } 50 reactions

$$c = (0.96 \pm 0.08) \cdot b$$

In these 50 Mantoux reactions there was thus no statistical difference in the control measurements, but neither can the hypothesis $b = 0.79\ a$ be excluded with certainty. These 50 reactions are taken at random, and should be considered representative of the total number of cases examined.

In this work the terminology »no difference», »probable difference», »very probable difference» and »statistically significant difference» is used, depending on whether the probability P, that the opposite result will be obtained in the same examination, is greater than 0.05, lies between 0.05 and 0.01, between 0.01 and 0.001, or is less than 0.001. The probabilities are obtained from the known *t-table* (Cfr. for instance CRAMÉR)

f	$P = 0.05$	$P = 0.01$	$P = 0.001$
10	t = 2,228	t = 3,169	t = 4,587
20	2,086	2,845	3,850
40	2,021	2,704	
60	2,000	2,660	
120	1,980	2,617	

in which the column furthest to the left gives the »degree of freedom», the values in the top line the probabilities, and the other values the quotient of the difference and the mean error of the difference.

The degree of freedom is an expression for the number of cases examined, in that if n_1 and n_2 represent the number of cases in the respective groups examined, the degree of freedom is

$$f = n_1 + n_2 - 2.$$

$$t = \frac{m_1 - m_2}{\sqrt{\varepsilon_1^2 + \varepsilon_2^2}}$$

In this expression m_1 and m_2 are the mean values of the different groups and ε_1 and ε_2 the error of the mean. If the number of cases is great — more than 120 — the t-table shows that the difference is »probable», »very probable» or »statistically significant» if it is 2, 2.6 or 3.4 times the mean error of the difference.

Parts of the skin with definite anatomical differences

Adults

In this section the author has mainly studied the role of the anatomical factors, especially that of the capillary system, in the mechanism of intracutaneous tuberculin reactions. For that reason areas of the skin, whose capillary density and depth were quite different, were selected for examination. These areas on the dorsal side of the forearm and on the back between the shoulder blades have been described previously in the section on Technique. They differ greatly in adults as to depth and capillary density, as mentioned.

The division of the material is shown on Page 34, and comprises 126 adult tuberculous patients and 109 children. The children are described separately.

The Mantoux reactions are described in the following with regard to dilution, age group, sex, and reading:

Table 4 (Page 46) shows that the Mantoux reactions on the skin of the arm, in different dilutions in the two sexes, and in all the age groups, are much milder than the reactions on the skin of the back. In the total number of cases there are actually only about 9 per cent of cases in which A (arm) is greater than R (back). As the surfaces R are greater than the surfaces A in about 91 per cent of the cases examined, calculation of the mean error was not considered necessary. In the different age groups there are small variations. In Fig. 1 this fact is shown graphically.

As the average in each age group of female cases is only 10 and of males 15, in the different age groups, no far-reaching conclusions can be drawn. There is however a tendency for the reactions to be milder in the higher age groups. The differences between A and R are not, on the other hand, influenced by age.

In Table 5 the different age groups are combined and in Table 6 the sexes are given in addition. It is found that A is on an average about 40 per cent less than R with 0.01 mg. Mantoux and about 60 per cent less with 0.001 mg. Mantoux. No sex difference is observed.

TABLE 4

The means (sq.mm.) of the Mantoux reactions on back (R) and arm (A), combined according to tuberculin dilution used, sex, age and hour of reading (I: 24 hours, II: 48 hours, III: 72 hours after the injection). R—A is the difference of the means in sq.mm.

Mantoux mg.	Sex	Reading	15—19 years			20—29 years			30—39 years			40—49 years			50—59 years			60—69 years		
			Number of cases																	
			7			18			12			9			3			1		
			R	A	R—A	R	A	R—A	R	A	R—A	R	A	R—A	R	A	R—A	R	A	R—A
0.01	♀	I	271.9	140.4	131.5	229.9	143.8	86.1	220.8	144.0	76.8	164.2	77.1	87.1	169.5	61.8	107.7	120.6	50.0	70.6
		II	261.2	165.2	96.0	248.2	164.8	83.4	244.1	151.3	92.8	176.4	122.5	53.9	227.1	74.6	152.5	253.6	204.5	49.1
		III	261.0	182.9	78.1	243.4	158.6	84.8	214.9	125.0	89.9	165.1	98.1	67.0	205.2	50.0	155.2	267.2	258.9	8.3
0.001	♀	I	53.3	10.1	43.2	58.0	19.4	38.6	88.2	15.0	73.2	36.7	13.2	23.5	17.6	0.0	17.6	0.0	0.0	0.0
		II	97.1	28.6	68.5	86.2	31.7	54.5	102.3	29.8	72.5	50.6	12.9	37.7	43.0	3.3	39.7	10.0	0.0	10.0
		III	77.3	25.0	52.3	95.7	36.2	59.5	104.4	39.3	65.1	53.5	11.5	42.0	42.9	60.3	−17.4	61.7	0.0	61.7
			Number of cases																	
						10			18			34			6			8		
0.01	♂	I				205.5	79.3	126.2	209.8	125.5	84.3	212.2	118.3	93.9	145.9	80.2	65.7	185.1	109.1	76.0
		II				217.7	112.8	104.9	243.1	136.5	106.6	200.5	131.1	69.4	182.0	102.4	79.6	219.8	125.3	94.5
		III				197.1	101.4	95.7	207.6	136.9	70.7	214.6	118.4	96.2	186.8	110.9	75.9	208.0	149.1	58.9
0.001	♂	I				63.9	32.9	31.0	53.3	21.8	31.5	71.4	21.2	50.2	57.5	28.6	28.9	60.2	27.7	32.5
		II				92.3	43.7	48.6	89.5	27.7	61.8	95.2	35.0	60.2	78.6	36.1	42.5	61.1	32.8	28.3
		III				84.0	43.0	41.0	94.6	31.0	63.6	96.4	33.7	62.7	75.4	31.7	43.7	68.9	44.9	24.0

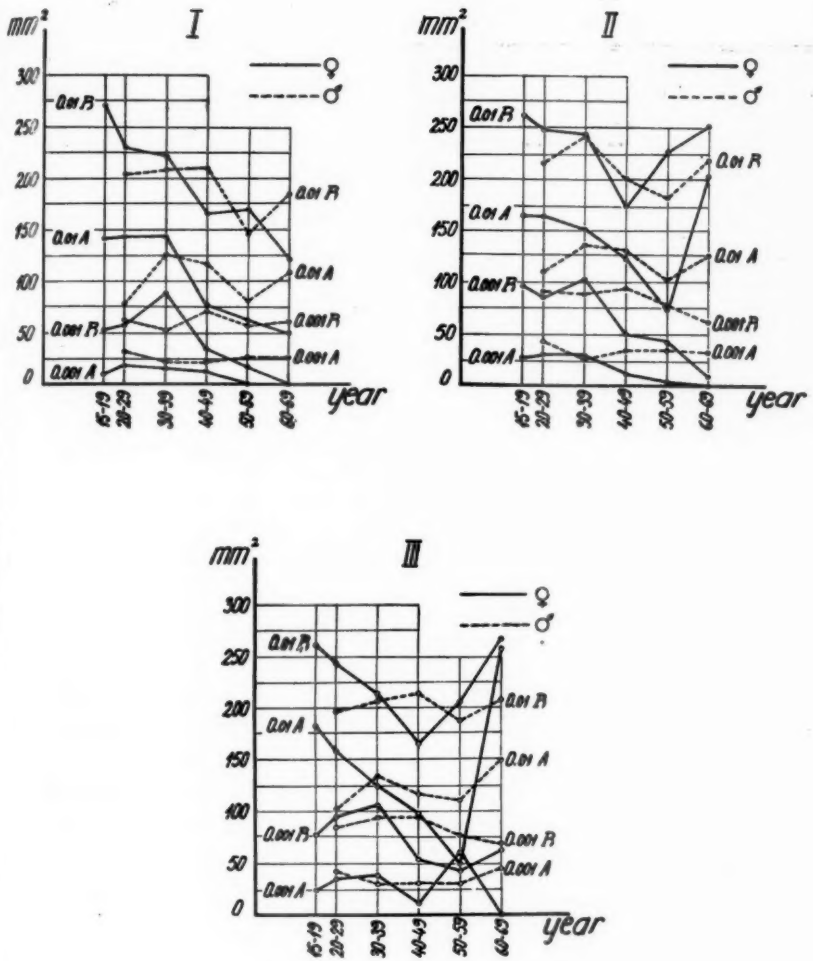


Fig. 1. Graphical presentation of the means (sq.mm.) of the Mantoux reactions on back and arm, combined according to tuberculin dilution used, sex, age and hour of reading.

TABLE 5

The means (sq.mm.) of the Mantoux reactions on back and arm, combined according to tuberculin dilution used, sex and hour of reading.

Mantoux mg.	Sex	Number of cases	24 hours			48 hours			72 hours			Mean of all readings		
			R	A	R—A	R	A	R—A	R	A	R—A	R	A	R—A
0.01	♀	50	216.0	124.6	91.4	235.0	149.4	85.6	223.1	138.5	84.6	224.7	137.5	87.2
	♂	76	202.7	110.9	91.8	213.4	127.1	86.3	207.8	123.2	84.6	208.0	120.4	87.6
	♀+ ♂	126	208.0	116.4	91.6	222.0	136.0	86.0	213.9	129.3	84.6	214.6	127.2	87.4
0.001	♀	50	57.2	14.4	42.8	81.1	25.1	56.0	83.8	31.7	52.1	74.0	23.7	50.3
	♂	76	63.9	24.2	39.7	88.6	34.3	54.3	89.8	35.3	54.5	80.8	31.3	49.5
	♀+ ♂	126	61.2	20.3	40.9	85.6	30.6	55.0	87.4	33.9	53.5	78.1	28.3	49.8

TABLE 6

The means (sq.mm.) of the Mantoux reactions on back and arm in 126 tuberculous patients. R and A give the mean of the results of three readings. The per cent of the difference is calculated from R.

Mantoux mg.	R	A	R—A	Per cent
0.01	214.6	127.2	87.4	40.7
0.001	78.1	28.3	49.8	63.8

In order to understand the difference between A and R, and so as to prevent the large surfaces from playing too large a part in the material, the mean of the percentages, which denote how many per cent A is less than R in the separate cases was calculated. The results are combined in Tables 7 and 8.

Mantoux mg.	Per cent
0.01	40.2
0.001	60.4

It is again seen that A is 40 per cent and 60 per cent, respectively, less than R to stronger, and weaker dilutions. The difference is de-

TABLE 7

The difference between each reaction pair (back and arm) in per cent calculated according to the expression $\frac{R-A}{R} \cdot 100$ *%. The mean is calculated on the readings in 126 patients, and combined according to sex, age and hour of reading.*

Mantoux mg.	Sex	Reading	15—19	20—29	30—39	40—49	50—59	60—69
			7	18	12	9	3	1
0.01	♀	I	53.0	30.2	31.2	49.0	60.2	58.5
		II	35.4	27.7	35.6	31.2	71.5	19.4
		III	30.4	30.8	38.8	42.2	62.3	3.1
0.001	♀	I	61.5	53.8	73.5	57.0	100.0	—
		II	24.2	58.8	70.0	73.9	66.7	100.0
		III	47.0	55.3	62.2	85.4	−18.0	100.0
			0	10	18	34	6	8
0.01	♂	I		64.6	39.2	47.2	50.0	48.4
		II		48.3	38.9	36.5	45.2	44.2
		III		46.0	32.4	43.9	38.9	32.7
0.001	♂	I		61.9	33.5	70.1	49.6	51.5
		II		59.9	66.8	67.4	77.9	66.5
		III		54.8	68.3	68.2	70.9	50.3

TABLE 8

The difference between each reaction pair (back and arm) in per cent calculated according to the expression $\frac{R-A}{R} \cdot 100$ *%. The mean is calculated on the readings in 126 patients and combined according to sex and hour of reading.*

Mantoux mg.	Sex	*n*	24 hours	48 hours	72 hours	Mean of all readings
0.01	♀	50	39.4	33.8	36.1	36.4
	♂	76	47.9	40.1	39.9	42.6
	♀ + ♂	126	44.5	37.6	38.4	40.2
0.001	♀	50	61.9	60.7	47.5	56.7
	♂	76	56.8	67.0	64.8	62.9
	♀ + ♂	126	58.8	64.5	57.9	60.4

pendent on the age, as seen; in the females there is a slight tendency to increase with the years.

Summarizing it may be said that the Mantoux reaction on the skin of the arm is much less (40 per cent, 60 per cent) than on the skin of the back, and that the difference R—A does not show any greater fluctuations than may be expected due to ordinary variations.

Children

The same examinations were made in children. Forty-two of the children were treated at the Kiljava Sanatorium. For practical reasons the reactions of these patients were read once only, 72 hours after the injection. The age distribution of the children examined is shown in the table.

Hospital	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	*Years*
Hospital for Tuberculous Diseases	3	27	12	9	7	5	—	2	2	—	—	—	—	—	—	67
Kiljava Sanatorium	—	—	—	—	—	8	3	3	8	3	3	3	3	3	5	42
Total	30		28			34					17					109

The mean of the tuberculin reactions in the cases are combined in the following tables according to dilution of tuberculin used, sex, age group and day of reading the result.

The results from children in the Hospital for Tuberculous Diseases is shown in Table 9 and from the Kiljava Sanatorium in Table 10. The reaction surfaces are decidedly less than in the adults. In addition a distinct variability is observed. Sometimes the difference R—A is positive, sometimes negative.

Calculating the mean error of the mean of the differences it is found that the means are so variable that conclusions, on the basis of these cases, cannot be drawn regarding the relation of size between R and A.

In Tables 11 and 12 the number of children is given, in which $R > A$, $R = A$ and $R < A$. Reactions designated $R = A$ denote

TABLES 9 and 10

The means (sq.mm.) of the Mantoux reactions on back and arm, combined according to tuberculin dilution, age and hour of reading. R—A is the difference of the means in sq.mm. n designates the number of cases, and Mean the mean of all readings.

I. Children. The Hospital for Tuberculous Diseases.

Mantoux mg.	Reading	0—2				3—5				6—10				0—10			
		n	R	A	R–A	*n*	R	A	R–A	*n*	R	A	R–A	*n*	R	A	R–A
0.01	I		108.8	71.1	37.7		135.7	128.9	6.8		184.1	137.9	46.2		130.2	104.6	25.6
	II	30	111.5	92.3	19.2	28	142.4	150.3	−7.9	9	175.6	130.9	44.7	67	133.2	122.1	11.1
	III		107.5	81.7	25.8		124.6	136.1	−11.5		172.9	149.0	23.9		123.4	113.8	9.6
0.001	I		31.0	20.2	10.8		47.4	36.3	11.1		54.3	48.0	6.3		41.1	30.7	10.4
	II	30	31.4	21.7	9.7	28	49.2	51.8	−2.6	9	65.0	53.7	11.3	67	43.4	38.8	4.6
	III		23.2	20.8	2.4		41.0	42.6	−1.6		54.2	43.8	10.4		34.9	33.1	1.8
0.01	I—III		109.3	81.7	27.6		134.2	138.4	−4.2		177.5	139.2	38.3		128.9	113.5	15.4
0.001	I—III		28.5	20.9	7.6		45.9	43.6	2.3		57.8	48.5	9.3		39.8	34.2	5.6

II. Children. The Kiljava Sanatorium.

Mantoux mg.	Reading	6—10				11—15			
		n	R	A	R—A	*n*	R	A	R—A
0.01	III	25	122.8	122.1	0.7	17	115.1	131.1	−16.0
0.001	III		45.4	34.2	11.2		49.4	45.5	3.9

TABLES 11 and 12

The number of reactions, combined according to tuberculin dilution used and hour of reading. The difference between the reaction pairs is 20 per cent.

1. Children. The Hospital for Tuberculous Diseases

Mantoux mg.	Reading	0–2				3–5				6–10				0–10			
		n	R>A	R=A	R<A	*n*	R>A	R=A	R<A	*n*	R>A	R=A	R<A	*n*	R>A	R=A	R<A
0.01	I		15	9	6		9	12	7		5	3	1		29	24	14
	II	30	12	10	8	28	6	12	10	9	6	3	0	67	24	25	18
	III		14	10	6		4	12	12		4	4	1		22	26	19
0.001	I		14	12	4		10	12	6		4	3	2		28	27	12
	II	30	11	15	4	28	6	11	11	9	4	4	1	67	21	30	16
	III		8	14	8		6	10	12		4	5	0		18	29	20
0.01	I+II+III		41	29	20		19	38	29		15	10	2		75	77	51
0.001	I+II+III		33	41	16		22	35	29		12	12	3		67	88	48

IV. Children. The Kiljava Sanatorium.

Mantoux mg.	Reading	5–10			10–15		
		R > A	R = A	R < A	R > A	R = A	R < A
0.01	III	6	13	6	3	8	6
0.001	III	9	11	5	7	6	4

that A differs by less than 20 per cent from R. It is observed that the groups are about equal in size. Group A = R is somewhat larger than the others. Group R > A is somewhat larger than group R < A; the relationship is approximately 3:2. Table 9 shows that the mean of A is about 13 per cent less than of R, a circumstance which agrees with the tendency already mentioned. The reactions in the several cases are so variable that all conclusions should be drawn with the utmost reservation.

Daily curves of tuberculin reactions

The tables given show that the mean of the reactions in the different readings do not reveal statistical differences between the sexes. Hence the groups are combined in the following.

Mantoux 0.01 mg.

Read (hours)	24	48	72	*Adults* $n = 126$
	Sq. mm.	Sq. mm.	Sq. mm.	
Arm	116.4	136.0	129.3	
Back	208.0	222.0	213.9	

Mantoux 0.001 mg.

Arm	20.3	30.6	33.9
Back	61.2	85.6	87.4

Mantoux 0.01 mg.

Read (hours)	24	48	72	*Children* (0—10 yrs) $n = 67$
	Sq. mm.	Sq. mm.	Sq. mm.	
Arm	104.6	122.1	113.8	
Back	130.2	133.2	123.4	

Mantoux 0.001 mg.

Arm	30.7	38.8	33.1
Back	41.1	43.4	34.9

The tables show that the differences between the reactions on the various days of reading are not great. In 0.01 mg. Mantoux reactions in adults the second day's reading is highest, but in 0.001 Mantoux reactions the third day's is highest. In the children the reactions are greatest on the second day. An approximate estimation of the mean error shows that the differences may be ascribed to statistical variations.

Parts of the skin with slight anatomical differences

The dependence of tuberculin reactions on anatomical factors of the skin has been shown in the preceding by comparison of Mantoux tests in the skin on the arm and on the back. The results are analogous and statistically significant. That the anatomy of the skin actually influences the size of the reactions to a considerable extent is supported by the fact that similar skin areas give similar reactions (Cfr. Mantoux double determinations).

In the following a small series is described which illustrates the results of Mantoux tests in skin areas in which the anatomical differences are slight (SPALTEHOLTZ 1893). The middle part of the forearm was chosen for this purpose and the dorsal and volar sides of the arm were compared.

Thirty-six tuberculous patients included in the previous series were given 0.01 mg. Mantoux in the volar side and 0.01 mg. in the dorsal side, using the technique already described. The reactions were read after 24, 48 and 72 hours. The mean of the reaction surfaces on the dorsal side is designated A and that on the volar side A_k.

TABLE 13

The mean (sq.mm.) of the surfaces of the reaction pairs. $A_k - A$ is the mean of the per cents which in each separate reaction pair shows in how many per cent A_k is greater than A.

Cases	Part of skin	24 hours	48 hours	72 hours
Men 20	A	127.0 sq.mm.	122.0 sq.mm.	114.0 sq.mm.
	A_k	147.4 »	130.9 »	126.0 »
	$A_k - A$	18.9 %	10.8 %	14.0 %
Women 16	A	162.4 sq.mm.	131.8 sq.mm.	165.9 sq.mm.
	A_k	174.7 »	136.6 »	173.5 »
	$A_k - A$	10.2 %	3.0 %	7.0 %
Total 36	A	142.7 sq.mm.	126.3 sq.mm.	137.0 sq.mm.
	A_k	159.6 »	133.4 »	147.1 »
	$A_k - A$	15.0 %	7.3 %	10.9 %

It is observed that A_k is on an average about 10 per cent greater than A. Calculating the mean error the following is obtained:

Cases		24 hours	48 hours	72 hours
Men	20	18.9 %±8.8 %	10.8 %±5.5 %	14.0 %±6.1 %
Women	16	10.2 %±8.2 %	3.0 %±3.9 %	7.0 %±4.4 %
Total	36	15.0 %±6.0 %	7.3 %±3.5 %	10.9 %±4.0 %

No statistical difference is observed when men and women are treated separately — as may be expected due to this material being small. A difference which may be characterized »very probable» is however obtained for the whole series of men and women.

The relation of repeated Mantoux reactions to different parts of the skin

When a tuberculin test is made after a certain interval, with the same dose and (generally) in the same part of the skin as on the first occasion, the term 'repeated tuberculin test' is used. The first and second tuberculin reactions vary in strength as a rule, and this question has been studied in a number of investigations. In this work the problem is studied especially with a view to finding out whether anatomical differences of the skin play a part.

BJØRNSTAD's (1941) conception is that the first test sensitizes the organism to react to the second test. In his opinion the first reaction seems to »pave the way» for the second one. Following this train of thought it might be possible, analogously with earlier observations in this investigation, that the skin on the back, having a less close capillary network, is better able to retain antibodies, locally, which act against the tuberculin, than the skin on the forearm, and thus the reaction would be proportionately stronger on the back in the repeated test.

In the Scandinavian literature the question of sensitization after repeated tuberculin tests has been treated in addition to BJØRNSTAD, in a large work by HONKANEN (1944). He gives an

Author	Year	No. of cases	Groups investigated	Interval (days) between tests	Tuberculin sensitivity
Pirquet test					
ELLERMANN and ERLANDSEN	1909	50	non-tuberculous patients	4—137	increased
KÖGEL	1912	37	cheesy cases with good clinical improvement	30 or more	increased
			cheesy cases with slow clinical improvement, or deteriorated conditions		decreased or unchanged
			fibrous cases with no T.b. in sputum		slow, weak
			mild cases with good healing		decreased
			fibrous cases with T.b. in sputum and good healing		increased
GRUNDT	1913	130	prognosis good	30	increased
			prognosis poor		decreased
PÄTIÄLÄ	1943	40	lupus (22 cases)	5	slightly increased
			healthy people (18 »)		more increased
Mantoux test					
ROZENBLAT	1913	20	clinically tuberculous children (9 »)	8	no differential diagnostic importance
			clinically non-tuberculous children (11 »)		no differential diagnostic importance
					No increase Uncertain Distinct Great
BESSAU and SCHWENKE	1914	129	active T.b. in children (13 »)	8	77.0 % 15.5 % 7.5 % — %
			probable T.b. in children (23 »)		65.0 » 22.0 » 13.0 » — »
			uncertain T.b. in children (48 »)		42.0 » 25.0 » 29.0 » 4.0 »
			no clinical evidence of T.b. in children (45 »)		4.5 » 15.5 » 60.0 » 20.0 »
PRINGSHEIM	1914	124	open T.b. in lungs (41 »)	8	78.5 » 7.2 » 11.9 » 2.4 »
			probable T.b. (30 »)		24.0 » 6.0 » 30.0 » 30.0 »
			uncertain T.b. (4 »)		— » 25.0 » 50.0 » 25.0 »
			no clinical evidence of T.b. (48 »)		12.5 » — » 15.8 » 41.7 »

MARCHIONINI	1929	137	active lupus (137 cases) controls (89 »)	8	unchanged or weaker (82.5 %) increased (17.5 %) unchanged (14.6 %) increased (85.4 %)
LOBBAN	1930	108	prognosis good prognosis poor stationary	30 or more	increased decreased unchanged
REISS	1932	203	non-tuberculous inactive pulmonary T.b. cases with clinical evidence of lupus (30 %)	8	negative (10 %) increased (43 %) unchanged (10 %) cachectic (37 %) negative (10 %) increased (53 %) cachectic (37 %) increased (21 %) unchanged (50 %) cachectic (29 %)
CVETKOVA	1934	174	cutaneous T.b. (various forms)	8	increased (61.6 %) unchanged (9.3 %) cachectic (29.1 %)
FORBES	1939	35	healthy people	7—21	increased
BJORNSTAD	1941	162	active and inactive T.b. non-tuberculous patients	7—14	increased (64 %)
HONKANEN 396 patients Pirquet tested 385 patients Mantoux tested	1944	781	active pulmonary. T.b. (255 cases) inactive T.b. (25 ») pleurisy (63 ») healthy people (278 ») BCG vaccinated patients (116 »)	14	unchanged or slightly decreased increased increased increased decreased

extensive survey of the literature and for that reason it is mentioned briefly in this paper. The use of repeated tuberculin tests for differential diagnosis in active and inactive tuberculosis has been specially studied.

The results of the various authors differ greatly owing to the differences in their materials and to completely different principles for assessing having been used. HONKANEN's investigation is however the largest (781 cases); comprising 25 patients with inactive tuberculosis, 255 with active tuberculosis, and 278 healthy people. The reactions were certainly read by medical corps sergeants, and nurses, and the erythema — which is the unstable factor of the tuberculin reaction — was measured after 24 and 48 hours, that is, before the erythema has been re-formed. However, this a comparative study, and as the same principles were followed through the whole investigation, the results may be considered quite correct, especially as the material is large and double tests were made (on both forearms) which reduces the technical error.

Repeated Mantoux tests were made in the present investigation in 41 adults with tuberculosis, for the purpose of finding out whether the different parts of the skin reacted differently to tuberculin in the following age groups:

Cases	20—29	30—39	40—49	50—59	Total
Women	7	6	4	—	17
Men	7	4	11	2	24
Total	14	10	15	2	41

The tuberculin tests were made with 0.01 mg. and 0.001 mg. Mantoux, and read 24, 48 and 72 hours after the injection. The repeated injection was given 14 days after the first one, in analogous parts of the skin. All the patients were under treatment for tuberculous lung processes.

As the surfaces in this material are very small in many instances, the difference in per cent between the reactions, each separately, has not been calculated (e.g. if surface I is insignificant and surface II is 30 sq.mm., a large and unreliable per cent is obtained). There-

TABLE 14

The mean of the first (I) and second (II) reaction in sq.mm. The interval between the first and second injection was 14 days.

Mantoux	Part of skin	Reading (hours)	First test (mean in sq.mm.)	Second test (mean in sq.mm.)	Per cent
0.01 mg.	Arm	24	103.1	136.1	+31.9
		48	121.9	120.6	− 1.1
		72	103.0	129.0	+25.2
	Back	24	191.1	238.1	+24.6
		48	188.4	233.4	+23.9
		72	198.2	209.3	+ 5.6
0.001 mg.	Arm	24	26.3	39.9	+51.7
		48	39.4	35.5	− 9.9
		72	33.5	39.5	+17.9
	Back	24	75.6	89.6	+18.5
		48	90.2	92.2	+ 2.2
		72	94.6	95.5	+ 1.0

fore the per cent figure in the table shows how many per cent the mean of the difference II and I is of the former surface, in other words, by how many per cent the mean of II exceeds the mean of I.

The differences in the values in Table 14 are great, and the variations hardly allow any far-reaching conclusions to be drawn. In general II seems to be greater than I. In the figures there is, however, a mean error of the mean of about 10 per cent which shows that there is an increase in the sensitivity, but its size cannot be exactly determined by this method.

Mantoux	Reading (hours)	I > II			I = II			I < II		
		A	R	%	A	R	%	A	R	%
0.01 mg.	24	6	8	17	13	15	34	22	18	49
	48	14	7	26	14	12	32	13	22	42
	72	10	12	27	13	13	32	18	16	41
0.001 mg.	24	5	10	18	19	13	39	17	18	43
	48	12	14	32	14	11	30	15	16	38
	72	8	15	28	14	14	34	19	12	38

For comparing the results in this investigation with those of HONKANEN the material was divided into three groups: I less than II, I equal to II, I greater than II. If I is equal to II, I differs from II by not more than 20 per cent.

The figure in the last column shows in how many per cent of all readings (both A and R) I is greater or smaller than II, or equally great. A satisfactory correspondence is observed on comparison of these results with HONKANEN's. In 155 patients with pulmonary tuberculosis he had, in readings at 48 hours, 30.3, 36.1 33.6 per cent. The corresponding figures at 24 hours were 12.9, 37.4 and 49.7 per cent. The first figure denotes a decreased reaction (I greater than II), the second an equally great reaction (difference $\leqq$ 20 per cent) and the third an increased reaction (I less than II). HONKANEN's results and those given in the table are thus almost similar.

In the author's own material it is seen that *the part of the skin does not affect the tuberculin sensitivity in repeated Mantoux tests, to any extent*, as seen from the following:

0.01 mg.	I > II	I = II	I < II	*0.001 mg.*	I > II	I = II	I < II
Arm	30	40	53	Arm	25	27	52
Back	27	40	56	Back	34	37	47

Furthermore, the tuberculin sensitivity in these patients with active tuberculosis remains unchanged in repeated tests, or is somewhat increased more often than decreased. Great increases and decreases in sensitivity occurred equally rarely, yet the great increases in the reactions were slightly more frequent.

HONKANEN, similarly as the majority of investigators studying these problems, observed that the sensitivity in repeated tuberculin tests on healthy people was stronger than in the first one (ELLERMANN and ERLANDSEN, BESSAU and SCHWENKE, PRINGSHEIM, MARCHIONINI, FORBES, PÄTIÄLÄ). In patients with active tuberculosis HONKANEN found an unchanged or slightly decreased sensitivity in repeated tests, and says: »eine kräftige Steigerung spricht gegen Aktivität». On close study of his series this conclusion hardly seems justified, as the difference remained below 20 per cent (status quo) in such large numbers (36.1 per cent), and the differences between the other two groups was so small (3.3 per cent)

that an increase and decrease in tuberculin sensitivity was equally frequent.

A more correct assessment of HONKANEN's results seems to be that repeated tuberculin tests increase the sensitivity in healthy people, and that weaker reactions denote the presence of an active tuberculous process. This corresponds to the results of the majority of other workers. Some investigators have observed a slight increase in the reaction sensitivity, yet much weaker than in healthy people (KÖGEL, GRUNDT, LOBBAN), in particular in cases of active pulmonary tuberculosis with favourable prognosis. In spite of the differences in the material and technique used in the investigations reported in the literature, HONKANEN's results are fairly similar, with this interpretation, and agree with those of the author as well, in that *the reaction to the repeated test is stronger than to the first one in patients with tuberculosis.* In this investigation no difference was observed between the skin on the arm and the back in repeated Mantoux tests.

Summarizing the first section it may be mentioned that the technical error in the Mantoux reaction was 15 per cent. The author shows 1) that the Mantoux reaction in analogous parts of the skin were similar 2) that the reactions in adults were definitely stronger in the skin on the back than on the forearm, and 3) that the reaction in children varied greatly. The difference in reactions on the dorsal and volar sides of the forearm were very small, only 10 per cent. Anatomical differences of the skin do not play a part in repeated Mantoux tests.

Further, a correlation seems to exist between infiltration and erythema in these series.

Theories of the aetiology of rheumatic infections

The nomenclature of the rheumatic diseases varies and is unreliable. Some conformity is, however, found in the ideas suggested in VIRKKUNEN's (1946) plan, which agrees with those of WILLIAMS (1932), and KALBAK (1942).

Scandinavian and German literature	*Anglo-Saxon literature*
Febris rheumatica	Rheumatic fever
Infectio rheumatica	Febris rheumatica
Polyarthritis rheumatica acuta	
Rheumatismus acuta	
Rheumatoid	
Infektartrit	
Septisk artrit	
Polyarthritis rheumatica chronica	Rheumatoid arthritis
Polyarthritis chronica progressiva (primaria s. secundaria)	Atrophic arthritis
	Chronic infectious arthritis
	Proliferative arthritis
Osteo-arthrosis deformans	Ostheoarthritis
Arthropathia deformans	Hypertrophic arthritis
Osteoarthritis deformans	Degenerative arthritis (Climacteric arthritis)

The aetiology of rheumatic infections has still not been clarified. At the end of the last century and at the beginning of this century, when great progress was achieved in the bacteriology, a specific agent was ardently sought after in the synovial fluid (effusion).

Several workers have reported bacterial findings of different kinds, but no conclusive evidence has been found in the control tests, and no specific rheumatic virus has thus been discovered.

Another discovery which for some time has given rise to speculations and scientific controversies was made by LÖWENSTEIN (1930). He propounded the view that rheumatic infection is caused by B. tuberculosis, which would support PONCET's and GROCCI's idea of »rheumatisme tuberculeux».

LÖWENSTEIN found tubercle bacilli in the blood, and effusions in the joints from patients with rheumatic fever, and REITTER and LÖWENSTEIN (1932) state: »der akute Rheumatismus ist eine exsudativ-entzündliche Phase im Ablauf einer tuberculösen Reinfektion».

A number of investigations were performed in the 1930's in order to confirm LÖWENSTEIN's reports. The question was discussed at the IX International Tuberculosis Congress in Tripolis in 1937. A symposium of the literature on this subject was made in 1940 by LAI and JORIO entitled »La bacillemia tubercolare nel reumatismo articulare».

Papers published	*No. of cases of polyarthritis examined*	*B. tub. cult. positive*	*B. tub. cult. negative*
70	1,961	580	1,381

The results obtained by LÖWENSTEIN and his direct or indirect collaborators were as follows:

	No. of cases	*Bacillaemia*
LÖWENSTEIN	144	22
REITTER and LÖWENSTEIN ..	88	56
POPPER	400	377
GRAZIOSI	24	12
	656	548 = 83.7 %
Other authors (65)	1,312	32 = 2.4 %

LAI and JORIO, themselves, report a series of 94 patients examined, of whom 40 had polyarthritis, and out of whom positive findings of tubercle bacilli were made in the blood in one case (2.5 per cent.). In 32 cases of »reumatismo tuberculare» there were no positive results.

In Scandinavia KAHLMETER (1932) and others have re-examined LÖWENSTEIN's reports with a view to confirming his results. The examinations were entirely negative. A monograph by SAENZ and COSTIL (1936) from the Pasteur Institute may be mentioned.

WISSLER (1946), who has studied especially the aetiology of rheumatoid arthritis in children, says that to-day no one contradicts the fact that more or less transient symptoms in the joints occur in connection with tuberculosis, without a specific arthritis arising. In opposition, all theories suggesting that rheumatic infection is a tuberculous disease as LÖWENSTEIN and his associates have tried to demonstrate — which is to »put tuberculosis and rheumatism under the same roof» (BERGER 1937) — have now been completely abandoned.

The well-known theory on focal infection has, on the other hand, increased the knowledge of the nature of rheumatic infection.

The theory now generally accepted regarding the aetiology of rheumatic diseases is the allergic, according to which the most important fact is the manner of reaction of the organism, not the factor which causes the manifested disease (KAHLMETER 1934). The theory was introduced by WEINTRAUD (1913), and later favoured in particular by RÖSSLE (1933), KLINGE (1936), JONSSON (1939), BRUUN (1940), and EDSTRÖM (1942).

The part played by the nervous system is emphasized especially in the so-called neurogenous theory by TRAUNER (1940). Some contributing factors have been mentioned, among them climatic, traumatic, hereditary (HOLSTI and RANTASALO 1936, and others).

In recent years rheumatoid arthritis has been explained as a sequel to adrenal-cortex-hormone-dysfunction (ACTH), HENCH (1949). Treatment with adrenal-cortex-hormone decreases the symptoms of rheumatoid arthritis (HENCH, KENDALL, SLOCUMB and POLLEY 1949).

II. The Mantoux reaction in rheumatoid arthritis

Material

In order to study the tuberculin sensitivity in rheumatoid arthritis, 125 adult patients were examined during the period April to November 1948 at the Sanatorium for Rheumatic Diseases at Heinola.

In addition, two control groups consisting of 130 tuberculous patients without rheumatic affections, and 130 healthy, tuberculin positive people were examined at the same time. Fifty-eight of the tuberculous patients were under treatment at the City of Helsingfors Hospital for Tuberculous Diseases, in either the department of the University or of the Commune, and 24 at the Kiljava Sanatorium. The remaining 48 were former in-patients of the Hospital for Tuberculous Diseases, who attended the hospital Outpatients for check-up of the lungs.

Similarly as in the cases in the first section (Page 34) of this investigation, these patients had tuberculous pulmonary lesions which were either fairly recent, or in the stage of healing. Febrile cachectic and weak patients were thus not tested in this examination.

The healthy people had all requested of their own accord to have their lungs examined at the Outpatients, or had been sent to the hospital in connection with mass radiography at their place of work. The tuberculin positive cases thus consisted of subjectively healthy people who had no symptoms of tuberculous or rheumatic disease.

Age and sex distribution of the cases examined is given in the table:

	Age-groups						Total	Average age	Standard deviation σ
	15—19	20—29	30—39	40—49	50—59	60—			
Tuberculous patients									
men	1	17	15	16	9	—	58	37.1	10.6
women ..	—	9	32	21	8	2	72	40.0	10.1
Healthy people									
men	1	18	12	10	9	1	51	36.5	11.5
women ..	4	24	25	18	8	—	79	34.5	10.4
Rheumatoid arthritics									
men	—	4	11	16	8	1	40	42.0	9.2
women ..	3	9	26	30	14	3	85	40.1	10.4

More women than men were examined in all groups, the ratio being:

	Men : Women
Tuberculous patients	0.81 : 1
Healthy people	0.65 : 1
Rheumatoid arthritics	0.47 : 1

All the rheumatic patients in the sanatorium were examined, except those who, from the history or clinically, had tuberculosis. As the material in the remaining groups was selected arbitrarily the ratio between men and women was not the same in all the groups; this should not, however, affect the results of the examinations (Page 69).

The age distribution in the various groups showed a majority of healthy people of the ages 20 to 40, whereas in the other groups the majority were between 30 and 50 years of age.

The average age and its distribution is shown in the table:

	Number of cases	Average age	Standard deviation σ
Tuberculous patients	130	38.7	10.3
Healthy people	130	35.5	10.9
Rheumatoid arthritics	125	40.7	10.1

Technique

All 385 people were subjected to the following examinations:

Tuberculin tests

Mantoux injections corresponding to 0.01 mg., 0.001 mg. and 0.0001 mg. old tuberculin (Behringwerke) were given in the skin of the back, between the shoulder blades (0.1 cc. of solutions 1:10,000, 1:100,000, and 1:1,000,000) according to the technique described previously. The reactions were read after 72 hours, in the day-light. The greatest diameter of the infiltration and a diameter perpendicular to it were measured. The reaction flush was estimated according to Tallqvist's haemoglobin colour-scale.

X-ray examination of the lungs

The tuberculous patients in hospital and under ambulatory control were all carefully examined by X-rays.

Radiographs of the size of 24 mm. × 24 mm. were taken of all the healthy people.

The X-ray control of the rheumatic patients afforded some difficulties which were solved by OY Yleisröntgen AB taking the pictures of the lungs, in size 24 mm. × 24 mm.

Sedimentation rate (BSR)

The sedimentation rate of the blood was tested by Westergren's technique which is commonly used in the Finnish hospitals. The results were read after 1 hour.

Antistreptolysin titre (AST)

By means of venous puncture 5 cc. of blood was taken and examined at the Sero-Bacteriological Institute of the University of Helsingfors according to their routine method (Oker-Blom 1947).

At the laboratory AST values up to 200 are considered border values between normal and pathological reactions, the technical error being ± 29 per cent (Oker-Blom 1949).

Pharyngeal test

A pharyngeal sample was taken with a sterile swab at the same time as BSR and AST were performed. The bacteriological examination for haemolytic streptococci was also done at the Bacteriological Institute. The swabs were kept in cardboard capsules which were taken to the laboratory not later than after 12 hours, so as to avoid drying and possible negative results (Rantasalo 1945).

The past history was recorded, particular note being taken of hereditary tuberculosis and tuberculous milieu, cured tuberculous or rheumatic diseases. In the case of the rheumatoids, possible infections before the rheumatic manifestations were inquired into, and all the patients were questioned regarding recent infectious diseases.

In addition to routine examination, special attention was paid to the correlation of tonsils and the teeth. The state of the joints of the rheumatic patients was recorded.

The Mantoux reaction in rheumatoid arthritics compared with that in tuberculous patients and healthy people

The mean of the surfaces of the tuberculin reactions and their mean errors in the different dilutions used, in tuberculous, healthy people, and rheumatic patients are given in the table:

	Mantoux 0.01 mg.	Mantoux 0.001 mg.	Mantoux 0.0001 mg.
	m±ε sq.mm.	m±ε sq.mm.	m±ε sq.mm.
Tuberculous patients			
men	235.0±11.9	72.5± 9.9	25.8±5.8
women	251.9± 8.5	73.0± 6.7	27.3±4.0
Healthy people			
men	206.1±11.9	69.5±10.0	26.6±4.8
women	167.1±10.3	66.1± 8.6	24.8±4.6
Rheumatoid arthritics			
men	129.9±13.1	18.9± 3.2	2.8±0.6
women	100.0±13.6	11.5± 2.1	2.0±0.5

(m = mean, ε = mean error)

Considering the mean errors, there is, in the healthy group only, tested with the strongest solution, a sex difference »very probable» ($P = 0.01$). In the rheumatic group, in the second dilution, there is the difference »probable» ($P = 0.05$) between the tuberculin reactions in men and women. There is no statistical difference in any of the other groups and as the difference in the groups mentioned is not »statistically significant» ($P < 0.001$), men and women may be regarded as the same in all the groups.

	Mantoux					
	0.01 mg.		0.001 mg.		0.0001 mg.	
	m±ε sq.mm.	σ	m±ε sq.mm.	σ	m±ε sq.mm.	σ
Tuberculous patients	244.2± 7.1	81.8	72.8±5.8	65.7	26.6±3.4	38.8
Healthy people	182.4± 8.0	91.2	67.5±6.5	74.2	25.5±3.3	37.6
Rheumatoid arthritics	109.6±10.2	113.7	13.9±1.8	20.1	2.2±0.4	4.7

The mean of the tuberculin reactions was compared between tuberculous, healthy and rheumatic people for each tuberculin dilution. In the tables above, values for t and P (— the possibility that the difference may be accidental) are given, except in two cases

The difference between the groups is shown below:

	Mantoux 0.01 mg.		Mantoux 0.001 mg.		Mantoux 0.0001 mg.	
	m ± ε sq.mm.	Difference	m ± ε sq.mm.	Difference	m ± ε sq.mm.	Difference
Tuberculous patients	244.2±7.1	D=61.8±10.7 t= 5.78 P< 0.001	72.8±5.8	5.3±8.7[1]	26.6±3.4	1.1±1.6[1]
Healthy people	182.4± 8.0	D=72.8±12.9 t= 5.64 P< 0.001	67.5±6.5	53.6±6.7 t=7.95 P<0.001	25.5±3.3	23.3±3.3 t=7.00 P<0.001
Rheumatoid arthritics	109.6±10.2		13.9±1.8		2.2±0.4	

No statistical difference is designated [1], the difference *D*, the coefficient from the *t*-table *t*, and the probability *P*.

in which it is evident that no statistical difference is present. *It is clearly seen that the tuberculin sensitivity in tuberculous and healthy people is stronger than in patients with rheumatoid arthritis.* Moreover, the reaction of the tuberculous to Mantoux 0.01 mg. is greater than that of healthy people.

In order to establish the representability of the material, a comparison may be made between the tuberculin sensitivity in the tuberculous patients in the first section and in the present section. The question thus concerns different test people in the two series, different technique of reading and, naturally, the clinical material is not homogeneous. However, the difference between the tuberculin sensitivity in the two groups is not great.

	No. of cases	*Mantoux 0.01 mg.*	*Mantoux 0.001 mg.*	*Read (hours)*
Tuberculous I . .	126	213.9	87.4	72
Tuberculous II . .	130	244.2	72.8	72

Studying the tables it is now seen that the tuberculin reactions are greater, as expected, in tuberculous patients than in healthy

people. This is however true only in the case of the stronger tuberculin dilution being used. In the other dilutions the tuberculin sensitivity is, as mentioned, the same. The figures which show the tuberculin sensitivity in the rheumatoids is, conversely, much lower than in the other groups. In these 125 patients with rheumatoid arthritis the capacity to react to intracutaneous injections of tuberculin in dilutions 1:10,000, 1:100,000 and 1:1,000,000 was thus on an average weaker than in healthy people and in the tuberculous patients. The reaction capacity, with regard to the weaker Mantoux dilution, was practically nil, similarly in the table below in which the number of very slight reactions in the different groups are given separately. An arbitrary figure, *40 sq.mm.*, has been taken for the border value. Reactions comprising 40 sq.mm. and larger are thus included in one group, the smaller reactions in another group. This size of reaction, with a diameter of about 6—7 mm., can be measured with fair accuracy, and it corresponds almost to what is clinically considered a positive tuberculin reaction (for diagnosis in children, WALLGREN's (1930) 10 mm. × 10 mm. flush and infiltration is used). In the following table the patients are grouped on the basis of weak tuberculin reactions:

		Mantoux 0.01 mg.		Mantoux 0.001 mg.		Mantoux 0.0001 mg.	
		sq.mm.		sq.mm.		sq.mm.	
		< 40	≥ 40	< 40	≥ 40	< 40	≥ 40
Tuberculous patients..	130	1	129	56	74	106	24
Healthy people	130	10	120	58	72	107	23
Rheumatoid arthritics	125	40	85	112	13	125	—

It is observed that the group of rheumatoids shows a very much greater number of small tuberculin reactions than the other test groups. By subdividing the series further the same relation is obtained.

Grouping of the patients on the basis of their tuberculin reactions is seen on the following page.

	Mantoux	0—39 sq.mm.	40—159 sq.mm	160—279 sq.mm.	280—399 sq.mm.	400— sq.mm.	Total number of cases
Tuberculous patients	0.01	10	43	55	20	2	130
	0.001	58	62	5	5	—	130
	0.0001	107	20	3	—	—	130
Healthy people	0.01	1	16	79	28	6	130
	0.001	56	63	10	1	—	130
	0.0001	106	21	2	1	—	130
Rheumatoid arthritics	0.01	41	46	33	5	—	125
	0.001	112	13	—	—	—	125
	0.0001	125	—	—	—	—	125

The sensitivity to 0.01, 0.001 and 0.0001 mg. Mantoux tests was weakest in the group of 125 patients with rheumatoid arthritis, as compared with the groups of 130 healthy people and 130 tuberculous patients. The reactions were specially weak with 0.001 and 0.0001 mg. in the rheumatoid arthritics.

A correlation was observed between the Mantoux infiltration and erythema in the tuberculous patients; in the healthy people the correlation was probable; in the rheumatoids there was no correlation.

The relation of secondary infections to the tuberculin sensitivity

As almost two-thirds of the cases examined consist of patients treated in hospital, and as secondary infections contracted in the hospitals are not uncommon, the author has endeavoured to clarify, in the following, whether trivial, secondary infections may possibly influence the tuberculin sensitivity in the different groups, as maintained by LANDORF (1938), MICHAILOV and LEMBERSKIJ (1939), KOENIGSBERGER (1940) and others quoted by ANZALONE and PAGANO.

In addition to examinations of the pharynx and the lungs BSR, AST and haemolytic streptococci were examined, as mentioned. The results in respect of the three last-mentioned examinations are divided as seen from the following table:

	Cases	AST m ± ε	BSR m ± ε	Haemol. strept.			Total
				+	++	+++	
Tuberculous patients	130	231±19.0	33.7±2.4	16	4	6	26
Healthy people	130	193±10.8	10.8±0.9	6	3	1	10
Rheumatoid arthritics ..	125	273±30.2	29.6±2.1	8	–	–	8

The AST reactions which were greater than 200 (200 *not* included) were:

AST > 200

Tuberculous patients ..	38.3 %
Healthy people	36.1 %
Rheumatoid arthritics .	48.0 %

The healthy people thus showed normal BSR and AST values. A somewhat greater per cent of patients than usual had, however, a higher titre than AST 200. The »healthy material» of some Scandinavian authors has between 4.6 and 26 per cent AST ≦ 200 (Oker-Blom 1947, Lagercrantz 1948 and Schone and Steen 1949).

The mean of BSR in the tuberculous and the rheumatoid arthritics was of the same order of value and distinctly increased. All this is due to the specific tuberculous and rheumatoid infection, respectively, that is evident, but the slightly raised AST mean in tuberculous patients also indicates that streptococcal infection is present. The occurrence of pathogenic haemolytic streptococci in the pharynx of 6 tuberculous patients also points in that direction.

The postulated occurrence of secondarily infected patients, particularly among the tuberculous, is not so great, to be compared with each other. This observation is further supported by the fact that in all three groups there were, in the objective examination of the pharynx, as many (11—13 patients) with follicular tonsillitis, tonsillitis, pharyngitis, etc.

Hence the possible connection between the sensitivity of infection of the pharynx and tuberculin should be studied separately for each group.

A. The Mantoux reaction in relation to AST and BSR

1. Tuberculous patients

a. *In relation to AST*

1) *Mantoux 0.01 mg.*	AST ≦ 100		AST 101—199		AST 200—399		AST > 400	
	Cases	sq.mm.	Cases	sq.mm.	Cases	sq.mm.	Cases	sq.mm.
Men	16	233.2	20	299.9	17	263.8	5	191.8
Women ..	24	268.0	19	230.8	19	255.2	8	252.4
	40	254.1	39	230.3	36	259.3	13	229.1

	AST ≦ 200		AST > 200	
	Cases	sq.mm.	Cases	sq.mm.
Men.....	36	231.4	22	247.4
Women ..	43	251.5	27	254.4
	79	242.3	49	251.2

2) *Mantoux 0.001 mg.*	AST ≦ 100		AST 101—199		AST 200—399		AST > 400	
	Cases	sq.mm.	Cases	sq.mm.	Cases	sq.mm.	Cases	sq.mm.
Men.....	16	77.2	20	54.6	17	101.0	5	52.0
Women ..	24	87.2	19	67.4	19	67.6	8	52.8
	40	83.2	39	60.8	36	83.4	13	52.5

	AST ≦ 200		AST > 200	
	Cases	sq.mm.	Cases	sq.mm.
Men.....	36	64.6	22	89.9
Women ..	43	78.4	27	63.2
	79	72.2	49	75.2

3) *Mantoux 0.0001 mg.*	AST ≦ 100		AST 101—199		AST 200—399		AST > 400	
	Cases	sq.mm.	Cases	sq.mm.	Cases	sq.mm.	Cases	sq.mm.
Men.....	16	19.1	20	19.7	17	42.0	5	19.4
Women ..	24	36.7	19	22.6	19	22.1	8	18.9
	40	30.0	39	21.1	36	31.5	13	19.1

	AST ≦ 200		AST > 200	
	Cases	sq.mm.	Cases	sq.mm.
Men.....	36	19.8	17	36.9
Women ..	43	30.5	19	21.1
	79	25.6	36	28.2

No statistical difference between the different groups is found, as the primary material shows that the mean error of the mean is so great that the difference between the groups is non-significant.

b. *In relation to BSR*

The mean for BSR in the 130 tuberculous patients was 33.7 ± 2.35 and in the rheumatoid arthritics 29.6 ± 2.08 mm./1 hour. Therefore the cases in these two groups are subdivided into cases more and less than 25 mm./1 hour.

1) *Mantoux 0.01 mg.*	BSR ≦ 25 mm.		BSR > 25 mm.		Mean of all cases	
	Cases	sq.mm.	Cases	sq.mm.	Cases	sq.mm.
Men	32	254.3	28	213.0	60	35.0
Women ..	33	269.1	37	238.0	70	252.7
	65	261.8±11.1	65	227.2±8.8	130	244.5

The difference (261.8 − 227.2) is 34.6 ± 14.2 and is »probable». The probability is 0.014.

2) *Mantoux 0.001 mg.*	BSR ≦ 25 mm.		BSR > 25 mm.		Mean of all cases	
	Cases	sq.mm.	Cases	sq.mm.	Cases	sq.mm.
Men	32	33.0	28	60.5	60	72.5
Women ..	33	77.1	37	68.5	70	72.6
	65	80.0±9.9	65	65.0±6.2	130	72.5

Difference 15.0±11.7.
Probability 0.2. No statistical difference.

3) *Mantoux 0.0001 mg.*	BSR ≦ 25 mm.		BSR > 25 mm.		Mean of all cases	
	Cases	sq.mm.	Cases	sq.mm.	Cases	sq.mm.
Men	32	25.0	28	26.7	60	25.8
Women ..	33	41.0	37	14.9	70	26.9
	65	33.0±5.0	65	19.9±4.5	130	26.4

Difference 13.1±6.7.
Probability ≈ 0.06. No statistical difference.

The AST variations do not affect the tuberculin sensitivity, but a group of patients with lower values for BSR (border value — BSR 25/1 hour) react on an average somewhat more intensively. This difference is hardly due to secondary or harmless infections. It is probably a result of the tuberculous process itself.

2. Healthy people

a. *In relation to AST*

1) *Mantoux 0.01 mg.*	AST ≦ 100		AST 101–200		AST 201–400		AST > 400	
	Cases	sq.mm.	Cases	sq.mm.	Cases	sq.mm.	Cases	sq.mm.
Men	9	216.1	22	206.4	15	199.6	5	206.4
Women	23	182.3	29	180.1	25	140.2	2	139.5
	32	191.8	51	191.4	40	162.5	7	187.3

The sex difference is not significant and the groups may thus be combined. As the number of cases exceeding 400 is small and the groups under the normal figure 200 are equivalent, the results over and under the normal value for AST, 200, may be compared.

	AST ≦ 200		AST > 200	
	Cases	sq.mm.	Cases	sq.mm.
Men	31	209.2	20	201.3
Women	52	181.1	27	140.1
	83	191.6	47	166.2

The difference (D) 25.4 ± 16.0 sq.mm. of the mean is not significant, $t = 1.59$, the probability (P) of the difference being accidental is ≈ 0.11.

2) *Mantoux 0.001 mg.*	AST ≦ 100		AST 101–199		AST 200–399		AST > 400	
	Cases	sq.mm.	Cases	sq.mm.	Cases	sq.mm.	Cases	sq.mm.
Men	9	71.6	22	70.5	15	74.0	5	47.8
Women	23	67.0	29	79.5	25	34.3	2	10.5
	32	68.3	51	75.6	40	61.7	7	37.1

The difference between groups 2 and 4 is:

AST 101–199	AST > 400
m = 75.6 sq.mm.	m = 37.1 sq.mm.
n = 51	n = 7

$D = 38.5 \pm 17.1$

The probability that the difference is accidental is 0.04.

However, as a comparison between the smallest and the greatest mean is not theoretically correct, the »normal group» AST ≦

200 and a group for high values AST $\geqq$ 400 are compared and the following results are obtained:

AST $\leqq$ 200 (n = 83)	AST $\geq$ 400 (n = 18)
72.8 $\pm$ 9.2 sq.mm.	49.8 $\pm$ 9.0 sq.mm.

$$D = 23.0 \pm 15.2$$
$$t = 1.51$$

The probability of the difference being accidental is 0.1, i.e. no statistical difference. Re-grouping into »normal AST» and »increased AST» may thus be done:

	AST $\leqq$ 200		AST > 200	
	Cases	sq.mm.	Cases	sq.mm.
Men	31	70.8	20	67.5
Women	52	74.0	27	51.0
	83	72.8	47	58.0

The difference 14.8 $\pm$ 12.2 sq.mm. is not statistically significant.

$$P \approx 0.23$$
$$t = 1.21$$

3) *Mantoux*	AST $\leqq$ 100		AST 101–199		AST 200–399		AST > 400	
0.0001 mg.	Cases	sq.mm.	Cases	sq.mm.	Cases	sq.mm.	Cases	sq.mm.
Men	9	20.3	22	31.5	15	24.4	5	22.6
Women	23	21.5	29	32.0	25	21.4	2	2.0
	32	21.2	51	31.8	40	22.6	7	16.7

The difference between AST 200 and AST 400 is:

AST $\leqq$ 200 (n=83)	AST $\geqq$ 400 (n=18)
27.7 $\pm$ 4.2	17.7 $\pm$ 6.0

The difference 10.0 $\pm$ 7.3 is not statistically significant.

	AST $\leqq$ 200		AST > 200	
	Cases	sq.mm.	Cases	sq.mm.
Men	31	28.3	20	24.0
Women	52	27.3	27	20.0
	83	27.7	47	21.7

$$D = 6.0 \pm 6.2$$
$$t = 0.96$$

The probability $\approx$ 0.35 is not significant.

It is thus observed that the variations in the values obtained for the AST titrations in the 130 healthy test-cases have not influenced the tuberculin sensitivity, performed with 0.01, 0.001 and 0.0001 mg. of old tuberculin.

b. *In relation to BSR*

The mean for BSR in the 130 healthy test-persons was 10.8 ± 0.88, therefore the following series is subdivided into groups of test-persons with BSR over, under, or equal to 10 mm./1 hour.

1) *Mantoux 0.01 mg.*	BSR ≦ 10 mm. Cases	sq.mm.	BSR > 10 mm. Cases	sq.mm.	Mean of all cases Cases	sq.mm.
Men	41	210.8	10	187.0	51	206.1
Women ..	46	170.4	33	162.5	79	167.1
	87	189.4±10.1	43	168.2±13.5	130	182.4
	D = 21.2 ± 16.8 and t = 1.26					
2) *Mantoux 0.001 mg.*						
Men	41	75.0	10	47.2	51	69.5
Women ..	46	66.0	33	66.3	79	61.4
	87	70.2±8.2	43	61.9±10.8	130	67.5
	D = 8.3 ± 13.5 and t = 0.61					
3) *Mantoux 0.0001 mg.*						
Men	41	26.9	10	25.5	51	26.6
Women ..	46	22.6	33	28.0	79	24.8
	87	24.6±4.2	43	27.4±5.5	130	25.5
	D = 2.8 ± 6.9					

Thus, the variations in the values for the sedimentation rate do not either influence the tuberculin sensitivity.

3. Rheumatoid arthritics

a. *In relation to AST*

1) *Mantoux 0.01 mg.*	AST ≦ 100 Cases	sq.mm.	AST 101—199 Cases	sq.mm.	AST 200—399 Cases	sq.mm.	AST > 400 Cases	sq.mm.
Men	10	101.8	9	116.9	14	142.0	7	162.3
Women	16	84.6	30	111.6	28	99.8	11	91.4
	26	91.2	39	112.8	42	113.9	18	118.9

	AST ≦ 200		AST > 200	
	Cases	sq.mm.	Cases	sq.mm.
Men	19	108.9	21	148.8
Women ..	46	102.2	39	97.4
	65	104.2	60	115.4

The difference is not significant.

2) *Mantoux*	AST ≦ 100		AST 101—199		AST 200—399		AST > 400	
0.001 mg.	Cases	sq.mm.	Cases	sq.mm.	Cases	sq.mm.	Cases	sq.mm.
Men	10	15.5	9	16.1	14	16.9	7	31.3
Women ..	16	15.2	30	10.0	28	11.3	11	10.5
	26	15.3	39	11.4	42	13.2	18	18.6

	AST ≦ 200		AST > 200	
	Cases	sq.mm.	Cases	sq.mm.
Men	19	15.9	21	21.7
Women ..	46	11.8	39	11.1
	65	13.0	60	14.8

The difference is not significant.

3) *Mantoux*	AST ≦ 100		AST 101—199		AST 200—399		AST > 400	
0.0001 mg.	Cases	sq.mm.	Cases	sq.mm.	Cases	sq.mm.	Cases	sq.mm.
Men	10	3.3	9	4.0	14	0.7	7	4.6
Women ..	16	1.3	30	3.4	28	0.9	11	1.9
	26	2.0	39	3.5	42	0.8	18	2.9

	AST ≦ 200		AST > 200	
	Cases	sq.mm.	Cases	sq.mm.
Men	19	3.6	21	2.0
Women ..	46	2.6	39	1.2
	65	2.9 ± 0.8	60	1.5 ± 0.6

$D = 1.4 \pm 1.0$
$t = 1.4$
$P > 0.1$

The difference is not significant.

b. *In relation to BSR*

1) *Mantoux 0.01 mg.*	BSR ≦ 25 mm. Cases	sq.mm.	BSR > 25 mm. Cases	sq.mm.	Mean of all cases Cases	sq.mm.
Men	20	145.4	20	114.3	40	129.9
Women ..	48	103.3	37	95.8	85	100.0
	68	115.7±10.2	57	102.3±12.6	125	109.6

The difference 13.4 ± 16.6 is not significant.

2) *Mantoux 0.001 mg.*	BSR ≦ 25 mm. Cases	sq.mm.	BSR > 25 mm. Cases	sq.mm.	Mean of all cases Cases	sq.mm.
Men	20	20.9	20	16.9	40	18.9
Women ..	48	13.9	37	8.3	85	11.3
	68	16.0±2.7	57	11.3±2.2	125	13.9

The difference 4.7 ± 3.5 is not significant.

3) *Mantoux 0.0001 mg.*	BSR ≦ 25 mm. Cases	sq.mm.	BSR > 25 mm. Cases	sq.mm.	Mean of all cases Cases	sq.mm.
Men	20	4.6	20	1.0	40	2.8
Women ..	48	2.3	37	1.5	85	2.0
	68	3.0±0.8	57	1.3±0.6	125	2.2

$D = 1.7 \pm 0.8$
$t = 1.75$
$P > 0.05$

The difference is not significant.

The shifts in the AST and BSR values in the series of tuberculous patients, healthy people, and rheumatoid arthritics do not affect the tuberculin sensitivity.

B. The Mantoux reaction in relation to bacteriological findings and clinical symptoms

A swab which was taken from each of the patients, was examined bacteriologically with respect to β-haemolytic streptococci. In addition the pharynx was inspected and the condition of the teeth observed. The lungs were auscultated with a view to finding acute or subchronic bronchitis. The glands of the

neck and the sub-mandibular glands were palpated. The incidence of infections is tabulated below:

	Haemolytic streptococci in pharynx				*BSR*	*AST*
Group examined	+	++	+++	*Total*	*Mean*	
Tuberculous patients	16	4	6	26	36.4	298.8
Healthy people	6	3	1	10	10.0	219.5
Rheumatoid arthritics	8	—	—	8	25.3	282.5
				44		

Patients with clinical symptoms of pharyngeal and upper respiratory passage infections:

	No. of patients	*Of these with haemol. streptococci*	*BSR*	*AST*
			Mean	
Tuberculous patients ..	11	4	23.4	300.5
Healthy people	11	1	18.5	265.5
Rheumatoid arthritics..	13	1	31.6	235.8

a. *β-haemolytic streptococci in the pharynx*

Tuberculous patients:

Pharyngeal findings: β-streptococci n = 26 — Pharyngeal findings: 0 n = 104

1) *Mantoux 0.01 mg.*

m = 218.4 ± 16.3 sq.mm. — m = 251.0 ± 8.0 sq.mm.

D = 32.6 ± 18.1
P = 0.07

2) *Mantoux 0.001 mg.*

m = 46.7 ± 8.5 sq.mm. — m = 79.0 ± 6.9 sq.mm.

D = 32.3 ± 11.0
P = 0.004

3) *Mantoux 0.0001 mg.*

m = 14.8 ± 9.1 sq.mm. — m = 29.2 ± 4.2 sq.mm.

D = 14.4 ± 10.0
P = 0.15

No statistically significant difference was thus observed between the two groups.

Healthy people:

Pharyngeal findings: β-streptococci n = 10	Pharyngeal findings: 0 n = 120
1) *Mantoux 0.01 mg.*	
m = 214.3 ± 38.5 sq.mm.	m = 179.7 ± 8.2 sq.mm.
2) *Mantoux 0.001 mg.*	
m = 89.9 ± 30.8 sq.mm.	m = 65.6 ± 6.5 sq.mm.
D = 24.3 ± 31.5	
3) *Mantoux 0.0001 mg.*	
m = 36.6 ± 13.7 sq.mm.	m = 24.6 ± 3.4 sq.mm.
D = 12.0 ± 14.1	

No statistically significant difference was thus observed between the two groups.

Rheumatoid arthritics:

Pharyngeal findings: β-streptococci n = 8	Pharyngeal findings: 0 n = 117
1) *Mantoux 0.01 mg.*	
m = 99.9 ± 26.4 sq.mm.	m = 110.2 ± 8.6 sq.mm.
D = 10.3 ± 27.8	
2) *Mantoux 0.001 mg.*	
m = 8.8 ± 2.0 sq.mm.	m = 14.2 ± 1.9 sq.mm.
D = 5.5 ± 2.8 P = 0.05	
3) *Mantoux 0.0001 mg.*	
m = 3.5 ± 1.7 sq.mm.	m = 2.1 ± 1.7 sq.mm.
D = 1.4 ± 2.4	

It is thus shown that the pharyngeal infection in healthy people does not influence the tuberculin sensitivity. In the tuberculous as well as in the rheumatoid arthritics the tuberculin allergy

in the non-infected seems to be somewhat lower with 0.001 mg. Mantoux. In the tuberculous the difference in this test was »very probable» and in the rheumatoid arthritics »probable».

b. *Clinical symptoms*

The diagnoses in these cases were: acute tonsillitis, chronic pharyngitis, follicular tonsillitis and acute or subchronic bronchitis.

Tuberculous patients:

Clinical symptoms n = 11	No symptoms n = 119
1) *Mantoux 0.01 mg.*	
m = 238.6 ± 16.9 sq.mm.	m = 245.1 ± 7.8 sq.mm.
D = 6.5 ± 18.6	
2) *Mantoux 0.001 mg.*	
m = 51.8 ± 12.4 sq.mm.	m = 74.4 ± 6.3 sq.mm.
D = 22.6 ± 13.9	
P > 0.1	
3) *Mantoux 0.0001 mg.*	
m = 16.4 ± 5.1 sq.mm.	m = 27.3 ± 3.6 sq.mm.
D = 10.9 ± 6.2	
P = 0.08	

No statistically significant difference was thus found between the two groups.

Healthy people:

Clinical symptoms n = 11	No symptoms n = 119
1) *Mantoux 0.01 mg.*	
m = 213.3 ± 27.1 sq.mm.	m = 179.5 ± 8.6 sq.mm.
D = 33.8 ± 23.7	

2) *Mantoux 0.001 mg.*

m = 95.9 ± 23.8 sq.mm. m = 64.8 ± 6.8 sq.mm.

D = 31.1 ± 24.7

3) *Mantoux 0.0001 mg.*

m = 36.4 ± 11.7 sq.mm. m = 24.5 ± 3.5 sq.mm.

D = 11.9 ± 12.2

No statistically significant difference was thus found between the two groups.

Rheumatoid arthritics:

Clinical symptoms n = 13	No symptoms n = 112

1) *Mantoux 0.01 mg.*

m = 151.1 ± 32.4 sq.mm. m = 104.7 ± 8.3 sq.mm.

D = 46.4 ± 33.5

2) *Mantoux 0.001 mg.*

m = 10.4 ± 5.3 sq.mm. m = 14.3 ± 1.9 sq.mm.

D = 4.3 ± 5.8

3) *Mantoux 0.0001 mg.*

m = 3.5 ± 1.6 sq.mm. m = 2.1 ± 0.5 sq.mm.

D = 1.5 ± 1.7

Findings of β-haemolytic streptococci in the pharynx, and clinical symptoms of secondary, harmless infections in the tonsils, pharynx and upper respiratory passage do not seem to affect the tuberculin sensitivity (no statistically significant difference) in these series of tuberculous patients, healthy people and rheumatoid arthritics.

Discussion

I

A number of factors co-operate in the origination of tuberculin reactions, as seen from the very extensive literature on tuberculin.

That the organism has been exposed to tuberculous infection is quite naturally the basal requirement. During an incubation time of on an average 4 to 6 weeks an antigenic-antibody system develops which causes a positive reaction to the tuberculin test. A local reaction occurs when the tuberculin comes into touch with the mucous membrane (ophthalmu or conjunctival reaction, and urethral reaction), subcutis (Косн) or skin.

The cellular tissue is the site of the visible, palpable, and histological reaction. The importance of the reticulo-endothelial system has previously been shown experimentally (Page 15). That the lymphatic vascular system co-operates in the development or the reaction is evident. The possible action of the blood capillary system is pointed out in this work. A close capillary system causes an accelerated transportation of blood from the skin, resulting in a weak tuberculin reaction. This is shown by the experiments reported in the literature. Circulatory stasis has been induced which causes stagnation of the capillary blood. No transportation takes place and the reaction becomes stronger. In analogy with this viewpoint, increased rate of circulation causes weaker reactions.

Some authors consider that hormonal and constitutional factors, in particular the autonomic nervous system, affect the mechanism that regulates the tuberculin reaction.

The author's results shed light on the importance of the skin — in particular depth and capillary density — for tuberculin sensitivity.

It has been found

— that Mantoux injections (0.01 mg.) in anatomically identical parts of the skin give reactions of the same strength;

— that the difference in the reactions (with 0.01 and 0.001 mg. Mantoux) is small when the injections are given in parts of the skin with slight anatomical differences;

— that the difference in the reactions is great if the injections are given in parts of the skin with great anatomical differences;

— that the difference is variable in children, and no definite tendency is observed.

II

The tuberculin allergy is changed in certain non-tuberculous conditions, which is briefly reported. The case is similar in rheumatoid affections.

The literature on tuberculin sensitivity in rheumatic fever and rheumatoid arthritic conditions is not homogeneous. Similarly as ROSENBERG, the great majority of authors have observed strong tuberculin allergy, not only in rheumatic fever, but also in rheumatoid arthritis.

YLPPÖ seems to be the only author who maintains that children with chronic polyarthritis have a specific immuno-biologic status. He has found children with evident tuberculous infection who, during a lengthy control period, have stubbornly remained negative to tuberculin. HELSINGIUS and YLPPÖ (1925), obtained more than 60 per cent positive Pirquet tests in children with rheumatic fever, and only 14 per cent in those with rheumatoid arthritis.

For the purpose of revealing the actual conditions in this respect the author studied the tuberculin sensitivity in 130 patients with rheumatoid arthritis. Tests with 0.01, 0.001, and 0.0001 mg. Mantoux were made, and the following results were obtained:

— in rheumatoids the reactions were very weak with all dilutions, in comparison with healthy people and tuberculous patients;

— secondary infections in the pharynx and upper respiratory passage did not affect the tuberculin allergy.

The results were distinct and similar.

Summary

The author has studied the technical error in the Mantoux reaction and has paid attention to the injection technique and reading. Double determinations were made with Mantoux 0.01 mg. in the skin on the back of 82 tuberculous patients, and the mean error 15 per cent was obtained. Different methods of measuring were tested (Petranyi's modification, and the usual perpendicular measuring) and it was found that the method generally used was quite reliable. The results were further checked by planimeter determinations.

The relation of the part of the skin to the tuberculin sensitivity was studied, and the following observations were made:

1. Mantoux tests in identical parts of the skin gave reactions of the same size (82 patients).

2. If the anatomical difference was small (forearm, volar and dorsal side) a slight difference was found (36 patients).

3. No difference was observed in reactions in the skin on the back or on the arm in children (109 patients).

4. In adults there was a marked difference (126 patients). The reaction in the skin on the back was much stronger than on the arm.

5. Tuberculin injections repeated after 14 days in adult tuberculous patients (41 patients) gave stronger reactions.

Furthermore, the author has studied the tuberculin sensitivity in patients with rheumatoid arthritis, and has found as follows:

1. The allergy to 0.01, 0.001 and 0.0001 mg. Mantoux was stronger in tuberculous patients (130 patients) than in healthy people (130 cases). The weakest reactions were observed in patients with rheumatoid arthritis (125 patients).

2. Harmless, secondary infections in the upper respiratory passage did not affect the tuberculin sensitivity.

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